Lab Medicine

Reviewer List

The *Laboratory Medicine* staff would like to extend a warm thank you to our 2020 *ad hoc* reviewers. We appreciate your time and dedication to the journal.

N. S. Abbai
Sherief Abd-Elsalam
Gorgy Abel
Bahattin Adam
Archana Agarwal
Muhammad Ahmad
Ahmad Akbari
Youssef Al-Sheikh
Mohammad Alam
Tom Alexander
Gunay Aliyeva
Loai Aljerf
David Alter
Muhammad Amjad
Marianna Antonelou
Sophie Arbefeville
Saja Asakrah
Veronica Balatti
Kayode Balogun
Daniel Bankson
Mustafa Barbhuiya
Laurie Bauer
Andrew Bellizzi
Mary Berg
Darci Block
J Francis Borgio
Anastasia Bougea
Kyle Bradley
Janetta Bryksin
Eileen Burd
Shaohang Cai
Jing Cao
Zheng Cao
Gerald Capraro
Analia Carmo
Simone Caruso
Gian Caviglia
Turgay Celik
Allison Chambliss
Thomas Chan
Kun Chen
Linfang Cheng
Jacky Chow
Lindsay Clark
Diana Cochran-Black
Jessica Colon-Franco
Stephen Cook
Laura Cooling
Elizabeth Courville
Genevieve Crane
Zhi-Jun Dai
Zunyan Dai
Thomas Daly
Saswati Das
Amitava Dasgupta
Phillip DeChristopher
Kristin Deeb
Meghan Delaney
Gregory Denomme
Stefanie Derer
Diana Desai
Habib Dhahari
Matthew Drake
Steven Drexler
Liat Drucker
Alexander Duncan
Paul Emery
Daping Fan
Matthew Feldhammer
Angela Ferguson
Marvin Jacob Fritzler
Koji Fujita
Li Gang
Chris Gardiner
Uttam Garg
Eric Gehrie
Shweta Gera
Jian-Long Guan
Rongjun Guo
Abdule Hafeez Kandhro
Joseph Hageman
Jens Andre Hammerl
Shuko Harada
Lara Harik
Karen Elise Heath
Richard Hodinka
Michael F Holick
Pedro Horna
Johnathan Hoyne
Biao Huang
Rongrong Huang
Ying Huang
Mina Hur
David Jaye
Fabrice Jean-Pierre
Dragan Jevremovic
Zhenzhou Jiang
Changzhong Jin
Zhicheng Jin
Wayne Jones
Floyd Josephat
Hala F. M. Kamel
Neena Kanwar
R. P. Kapur
Cem Karadeniz
Shinichiro Kashiwagi
Peter Kavsak
Steven Kazmierczak
Sue Kehl
Christopher Koch
Aaron Kofman
Nurhan Koksal
Milena Kordalewska
Alexandra Kovach
Matthew Krasowski
Attila Kumanovics
Shaji Kumar
Mei I Lai
Samuel Lalmuanawma
Seied Amir Latifi
Chris Lehman
Guang Lei
Chunliang Li
Xiaoxian Li
Michael Linden
Kelly Lockeman
Sanam Loghavi
Lesley Lomo
Pedro Luiz Toledo de Arruda Lourencao
Jian Lu
Veronica Luzzi
Masamitsu Maekawa
Etienne Mahe
Giulio Marino
Rahul Matnani
K. E. McMartin
Allison McMullen

Michele Merli
Ryan Metcalf
Gerald Miller
Farzaheh Moghaddam
Taher Mohiuddin
Alejandro Molinelli
Emily W. Moody
Gregers Mortensen
Karen Moser
Khushboo Munir
Lacey Murphy
Charles Myers
Megan Nakashima
Tina Netzker
Ajay Nooka
Seiichi Okabe
Min Seon Park
Marcela Pasetti
Khushbu Patel
Lauren Pearson
Erin Peckham-Gregory
Lisa Peterson
Maria Picken
Massimo Pieri
Fred Plapp
Iva Pruner
Amy Pyle
Phillipp Raess
Rema Rao
Jesus Reine
Gary Reisfield
Lacy Reynolds Moss
Marian Rollins-Raval
Manuel Romero-Gomez
Flavia Rosado
Joseph Rudolf
Henk Russcher
Max Salfinger
Natasha Savage
Debra Saxe
Sandeep Saxena
Hubert Scharnagl
Robert Schmidt
John Schmitz
Audrey Schuetz
Joseph Servadilo
Ratti Ram Sharma
Hao Shen
Gyorgy Simon
Deepika Sirohi
Melissa Snyder
Jerry Squires
Douglas Stickle
M. Sugimoto
Cheng-Cao Sun
Steven Suvalsky
Anne Tebo
Elitza Theel
Ciprian Tomuleasa
Tao-Hsin Tung
Tatiana Tvrdik
Frances Valencia-Shelton
Maria Vergara-Lluri
Pieter Vermeersch
Mrigender Virk
Anne Walsh-Feeks
Matthew Ward
Pamela Ward
Lars Westblade
Sarah Wheeler
Clayton Wilburn
Maria Alice Willrich
Kristy Wolniak
Yuansheng Xie
Lili Xiong
Xinjie Xu
Yi-Qing Yang
Xin Yi
Sophia Yohe
Brittany Young
Aykan Yucel
Linsheng Zhang
Meilan Zhu
Zhenjian Zhuo **LM**

Special Report

Call for Action: Journals Need to Insist on Full Reporting of the Analytical Characteristics of Biomarkers

Zhen Zhao, PhD,[1] *David B. Sacks, MB, ChB*[2*]

Laboratory Medicine 2021;52:7-9

DOI: 10.1093/labmed/lmaa097

Keywords: biomarker, analytical characteristics, guideline, reporting, reproducibility, transparency

There is a growing movement to encourage the publication of complete methods and to report the quality of data in basic research.[1-3] High-profile journals such as *Science* and *Nature* have published and endorsed several guidelines and checklists to promote transparency and reproducibility.[3-5] Although these guidelines place considerable emphasis on the transparent reporting of statistics, analytical validation has not been addressed. It is important to be aware that failure to adhere to appropriate methodological standards can lead to biased and misleading results, even when suitable statistical methods are applied.[6,7]

The lack of transparent, adequate reporting of analytical method validation may undermine credibility and reduce the relevance of a clinical study. Moreover, analytical shortcomings may be a barrier to generating reproducible results and translating important initial findings into clinical practice. Nevertheless, there has been no objective information available on the extent of characterization or reporting of the analytical performance of chemical biomarkers in published clinical studies. Therefore, we assessed the reporting of analytical characteristics of biomarkers used in clinical research and evaluated the extent of reported data on assay precision.[8]

Abbreviations:

FDA, U.S. Food & Drug Administration; STARD, Standards for Reporting of Diagnostic Accuracy.

[1]Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, New York, [2]Department of Laboratory Medicine, National Institutes of Health, Bethesda, Maryland

*To whom correspondence should be addressed.
david.sacks2@nih.gov

We analyzed all the clinical studies that were published in 5 high-impact medical journals: the *Annals of Internal Medicine, JAMA: The Journal of the American Medical Association, The Lancet, The New England Journal of Medicine,* and *PLOS Medicine,* over a 10-year period. Using the term "biomarker" as a search criterion, we identified 544 studies (with 1299 biomarkers) in which biomarkers were used for patient classification or inclusion/exclusion criteria or as a study outcome. For each study, we systematically reviewed the reporting of 11 analytical validation characteristics and the quality of validation. The key characteristics included analytical accuracy or trueness, imprecision, analytical sensitivity, interferences, reportable range, reference interval, cutoff or decision limits, quality control, and calibration.

Remarkably, our analysis revealed that the median reporting rate was zero. No characteristic was provided for 67% of the biomarker measurements, and more than 4 characteristics were reported for only 3% of the biomarkers. We also examined the completeness, comprehensiveness and quality of the reporting using total imprecision as an example because it showed the highest reporting rate at 13%. Of the 274 articles that reported imprecision, 76% reported only a total coefficient of variation, with no details about the concentrations measured or the number of days of testing. These findings suggest that even for the little information that was reported, the quality of method validation was far from adequate to meet expectations for the clinical use of biomarkers.

Values for a given patient sample can vary significantly between assays from different manufacturers (even for methods approved by the U.S. Food & Drug Administration) because of a lack of assay harmonization. Nevertheless, a persistent

problem we observed was a failure to report the name of the manufacturer, which was detected for 53% of biomarker measurements. As an illustration, we carefully examined the 2 biomarkers most commonly measured in the publications we assessed. The manufacturers of cardiac troponin (63 reports; 5%) and glucose (47 reports; 4%) methods were stated in only 35% and 33% of the studies, respectively. Cardiac troponin I results can vary up to 33-fold among assays from different manufacturers.[9] Similarly, glucose results can vary substantially among methods, especially between point-of-care glucose meters and central laboratory methods. Moreover, even if the manufacturer remains unchanged, methods can change over time with improved and/or more standardized measurements. Clinical studies can last many years, be performed at multiple locations, and use methods from different manufacturers. Therefore, documenting the assay method and keeping track of any changes to the method are essential to ensure the consistency of the measurement over the course of a study.

Analytical method validation by clinical laboratories is required in the United States by the Clinical Laboratory Improvement Amendments. Unfortunately, this concept has received minimal attention in the general scientific community. In basic and clinical research settings, investigators often assume that analytical validation and method optimization are the manufacturers' responsibility. However, this guideline pertains only to methods that have been approved by the FDA. Only 24% of the biomarker measurements in our study used FDA-approved methods. "Research Use Only" assays, which have not been cleared or approved by the FDA, were commonly used in the publications we examined. Although they are acceptable for clinical research, it is important to realize that these assays have not undergone analytical validation as rigorous as those of FDA-approved methods.[10] Therefore, it is essential that the pertinent validation be performed before the assays are used in clinical studies.

In 2003, the Standards for Reporting of Diagnostic Accuracy (STARD) Initiative developed a checklist for the complete and accurate reporting of diagnostic accuracy studies.[11] However, emphasis in STARD is placed on the reporting of clinical rather than analytical performance of methods. Clinical performance pertains to how results correctly classify patients and correlate with a patient's disease state and outcome. By contrast, analytical validation ensures an accurate and reproducible measurement, which serves as the foundation of clinical evaluation.[12] The only analytical validation item in STARD is cutoff. Notwithstanding this requirement, our evaluation revealed that only 6% of the 1299 biomarker measurements we assessed reported the cutoff. This poor adherence indicates that journals have not implemented STARD in the editorial and peer-review process as rigorously as they should. Our findings are congruent with 2 recent reports suggesting that although more than 200 biomedical journals have endorsed STARD, adherence to guidelines remains suboptimal.[13,14]

Although multiple factors contribute to the lack of reproducibility of clinical research, our findings specifically highlight how inadequate reporting of analytical characteristics can create barriers to study replication. An important contribution of our work is the opportunity to challenge the field to move beyond the current requirements of reporting the analytical items used in clinical studies. It has been suggested that standards for design and measurement would make clinical trials reproducible and usable.[15] Because numerous biomarker measurements have not been standardized, we recommend that when biomarkers are defined in clinical guidelines and used in clinical trials, basic analytical information such as cutoff, methods, and manufacturers should be provided to facilitate the interpretation and generalization of results to clinical practice. Although word limits could preclude detailed information regarding the methods, this information could readily be included in supplemental materials.

We also encourage enhanced communication between clinical laboratory staff and clinicians to raise awareness of the underreporting issues. The clinical community should come together to provide consensus recommendations on more informative reporting standards for analytical methods and biomarkers. Such guidelines could serve as a template for investigators to reduce analytical error when designing studies, for clinicians to better evaluate the analytical performance of assays before implementing them in patient care, for funding bodies to ensure the quality of data, and for other scientists to reproduce the biomarker measurements.

We recognize that there are other important items beyond the establishment and enforcement of guidelines/checklists that need to be addressed. First, research scientists often receive little training in the analytical and regulatory aspects of method development and validation. Second, there is no universal approach to validating analytical methods. The extent of analytical rigor need not be applied equally to discovery, initial exploratory studies, clinical trials, and clinical practice. Therefore, in an effort to develop a guideline for authors, a checklist that meets the purpose of the tests should be considered, along with the FDA-approval status and quantitative nature of the tests.

In summary, we have identified a previously underappreciated deficiency in the published clinical literature. The lack of reporting of analytical parameters of biomarkers is a threat to the interpretation and replication of clinical studies. Our analysis raises awareness and highlights an important limitation of many clinical studies. "How one measures matters," wrote Patrick Bossuyt, professor of clinical epidemiology at the University of Amsterdam, in an editorial in *Clinical Chemistry*[16] that commented on our study. He also perceived the results of our study as a call for action for clinical laboratory staff to request sufficient details about methods when reviewing manuscripts and commenting on study protocols. Our results emphasize the need for the development of "fit-to-purpose" guidelines that are easy for researchers, funding agencies, journals, and other stakeholders to follow. Additional solutions include mentoring young investigators on analytical rigor and transparency. Implementation of these strategies should enhance thorough and transparent reporting, which will enable readers to assess the quality of the work, evaluate the validity of the results, repeat the experiments, and ultimately decrease irreproducibility in future clinical research. **LM**

Acknowledgments

DBS is funded by the Intramural Research Program of the National Institutes of Health.

References

1. Iqbal SA, Wallach JD, Khoury MJ, Schully SD, Ioannidis JP. Reproducible research practices and transparency across the biomedical literature. *PLoS Biol.* 2016;14(1):e1002333.
2. Baggerly K. Disclose all data in publications. *Nature.* 2010; 467(7314):401.
3. Nosek BA, Alter G, Banks GC, et al. Scientific standards. Promoting an open research culture. *Science.* 2015;348(6242):1422–1425.
4. Announcement: towards greater reproducibility for life-sciences research in Nature [editorial]. *Nature.* 2017;546(7656):8.
5. Announcement: transparency upgrade for nature journals [editorial]. *Nature.* 2017;543(7645):288.
6. Lijmer JG, Mol BW, Heisterkamp S, et al. Empirical evidence of design-related bias in studies of diagnostic tests. *JAMA.* 1999;282(11): 1061–1066.
7. Riley RD, Abrams KR, Sutton AJ, et al. Reporting of prognostic markers: current problems and development of guidelines for evidence-based practice in the future. *Br J Cancer.* 2003;88(8):1191–1198.
8. Sun Q, Welsh KJ, Bruns DE, Sacks DB, Zhao Z. Inadequate reporting of analytical characteristics of biomarkers used in clinical research: a threat to interpretation and replication of study findings. *Clin Chem.* 2019;65(12):1554–1562.
9. Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. *Clin Chem.* 2012;58(11):1574–1581.
10. Jennings L, Van Deerlin VM, Gulley ML; College of American Pathologists Molecular Pathology Resource Committee. Recommended principles and practices for validating clinical molecular pathology tests. *Arch Pathol Lab Med.* 2009;133(5):743–755.
11. Bossuyt PM, Reitsma JB, Bruns DE, et al.; Standards for Reporting of Diagnostic Accuracy Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. The Standards for Reporting of Diagnostic Accuracy Group. *Croat Med J.* 2003;44(5):635–638.
12. Zhao Z, Sacks DB. Assay development in clinical pathology. In: McManus LM, Mitchell RN, eds. *Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms*. London, UK: Elsevier; 2014:3194–3206.
13. Korevaar DA, Wang J, van Enst WA, et al. Reporting diagnostic accuracy studies: some improvements after 10 years of STARD. *Radiology.* 2015;274(3):781–789.
14. Dilauro M, McInnes MD, Korevaar DA, et al. Is there an association between STARD statement adherence and citation rate? *Radiology.* 2016;280(1):62–67.
15. Dickersin K, Mayo-Wilson E. Standards for design and measurement would make clinical research reproducible and usable. *Proc Natl Acad Sci U S A.* 2018;115(11):2590–2594.
16. Bossuyt PM. Laboratory measurement's contribution to the replication and application crisis in clinical research. *Clin Chem.* 2019;65(12): 1479–1480.

DOI: 10.1093/labmed/lmaa097

Review

Thrombocytopenia Is Associated with COVID-19 Severity and Outcome: An Updated Meta-Analysis of 5637 Patients with Multiple Outcomes

Xiaolong Zong, MD,[1,2,#] *Yajun Gu, PhD,*[3,#] *Hongjian Yu, MD,*[4] *Zhenyu Li, PhD,*[2,5] *Yuliang Wang, PhD*[1*]

Laboratory Medicine 2021;52:10-15

DOI: 10.1093/labmed/lmaa067

ABSTRACT

The COVID-19 pandemic is persistent worldwide. A prior meta-analysis suggested the association of thrombocytopenia (TCP) with more severe COVID-19 illness and high mortality. Considering newly published studies, we updated the previous meta-analysis to confirm and explain the association of TCP with COVID-19 severity and multiple outcomes. Twenty-four studies with 5637 patients with COVID-19 were included in this study. The weighted incidence of TCP in COVID-19 was 12.4% (95% confidence interval [CI], 7.9%–17.7%). Data synthesis showed that the platelet number was lower in patients with either more severe illness or poor outcomes and even lower in nonsurvivors, with weighted mean differences of -24.56×10^9/L, -22.48×10^9/L, and -49.02×10^9/L, respectively. The meta-analysis of binary outcomes (with and without TCP) indicated the association between TCP and 3-fold enhanced risk of a composite outcome of intensive care unit admission, progression to acute respiratory distress syndrome, and mortality (odds ratio [OR], 3.49; 95% CI, 1.57–7.78). Subgroup analysis by endpoint events suggested TCP to be significantly associated with mortality (OR, 7.37; 95% CI, 2.08–26.14). Overall, the present comprehensive meta-analysis indicated that approximately 12% of hospitalized patients with COVID-19 have TCP, which also represents a sign of more severe illness and poor outcomes.

Keywords: coronavirus disease 2019, COVID-19, SARS-CoV-2, platelet, thrombocytopenia, prognosis

The coronavirus disease 2019 (COVID-19) pandemic has affected more than 12 million people and caused more than 500,000 deaths worldwide.[1] Although most patients develop mild or uncomplicated illness, those with older age and pre-existing comorbidities are more susceptible to severe disease and have a higher risk of poor outcomes such as admission to the intensive care unit (ICU), progression to acute respiratory distress syndrome (ARDS), and even death.[2,3] Thrombocytopenia (TCP) is commonly associated with viral infections and serves as a sign of sepsis progression and exacerbation.[4,5] A prior meta-analysis suggested that low platelet count was associated with high mortality and more severe COVID-19 illness.[6] Because new studies have been published, we conducted an update of the previous meta-analysis to confirm and explain the association of platelet number with COVID-19 severity and provide a direct comparison of the risk of multiple endpoints in patients with COVID-19 with and without TCP.

Abbreviations:

TCP, thrombocytopenia; CI, confidence interval; OR, odds ratio; ICU, intensive care unit; ARDS, acute respiratory distress syndrome; WMD, weighted mean difference; TPO, thrombopoietin; ITP, immune thrombocytopenia.

[1]Department of Laboratory, The Second Hospital of Tianjin Medical University, Tianjin, China, [2]Tianjin Key Laboratory of Ionic-Molecular Function of Cardiovascular Disease, Tianjin, China, [3]School of Medical Laboratory, Tianjin Medical University, Tianjin, China, [4]Department of Laboratory, Jinnan Hospital, Tianjin, China, [5]Intensive Care Unit, The Second Hosptial of Tianjin Medical University, Tianjin, China

*To whom correspondence should be addressed.
wang_yu_l@163.com

[#]*Xiaolong Zong and Yajun Gu contributed equally to this work.*

Materials and Methods

This systematic review was performed following the PRISMA statement (see Supplemental Material 1).[7] The study protocol is provided in Supplemental Material 2. Briefly, PubMed, Embase,

and Web of Science were searched to identify studies between December 1, 2019, and March 15, 2020, without language restriction. The following terms were used: "COVID-19" OR "Corona Virus Disease 2019" OR "coronavirus disease-19" OR "severe acute respiratory syndrome coronavirus 2" OR "SARS-COV-2" OR "2019 novel coronavirus" OR "2019-nCoV" OR "new coronavirus pneumonia." Because new articles on COVID-19 are daily published, eligible articles published between March 15, 2020, and April 18, 2020, were also identified via PubMed using the aforementioned terms with AND for the following terms: "platelet" OR "thrombocytopenia" OR "thrombo*" OR "hemato*." Studies were included in this meta-analysis if they met the following criteria: (i) adult population (older than 18), (ii) COVID-19 was confirmed by laboratory testing, (iii) platelet count or TCP frequency was reported, and (iv) English or Chinese full text was available. Studies involving patients with a particular illness or emergency conditions were excluded (eg, cancer and cardiovascular attack). When studies had significant overlapping data, the most comprehensive study was included.

For pooled analysis, data on platelet count and TCP frequency in the overall population and subgroups based on disease severity or outcomes were extracted by 2 independent investigators. COVID-19 severity was defined as per the World Health Organization or local interim guidance, and studies without definite criteria were excluded. We used a composite of admission to ICU, progression to ARDS, or all-cause mortality to define adverse outcomes of COVID-19. Double-arcsine-transformed proportion, weighted mean difference (WMD), and odds ratio (OR) with 95% confidence interval (CI) were calculated and pooled in the meta-analysis as appropriate. In their absence, the mean and standard deviation of the platelet count were estimated from sample size, median, and interquartile range.[8] A random-effects model was selected to account for clinical heterogeneity. Subgroup analyses were conducted based on endpoint events and TCP definition. The quality of the included studies was evaluated by 2 independent reviewers following the Newcastle-Ottawa scale (see Supplemental Material 3: Table 1). Discrepancies in data extraction and quality assessment were resolved through discussion with a third author. Statistical analyses were performed using the RStudio software.

Results and Discussion

After initial screening and full-text review, 24 studies[2,3,9-30] with 5637 patients with COVID-19 were included in this systematic review. The study selection procedure is illustrated in Supplemental Material 3: Figure 1; the excluded studies and reasons for exclusion are detailed in Supplemental Material 3: Table 2. **Table 1** shows the quality and general characteristics of the included studies. Seventeen studies[2,3,9-13,15,17–19,21,24,26–29] including 4671 patients reported the incidence of TCP in hospitalized patients with COVID-19 (see Supplemental Material 3: Figure 2). Our meta-analysis indicated a weighted incidence of 12.4% (I^2 = 95%; 95% CI, 7.9%–17.7%; **Table 2** and Supplemental Material 3: Figure 3). This result was consistent with that of a previous meta-analysis of 218 patients that reported a combined estimation of 11.5%.[31] Notably, the TCP frequency across included studies is inconsistent, with a wide range from 0% to 36.5%. This inconsistency may be attributed to different criteria applied for defining TCP and the heterogeneous disease severity spectrum among study populations. The results of subgroup analysis by cutoff values of 150, 125, and 100 × 109/L for TCP were 28.7% (95% CI, 15.6%–43.9%), 17.3% (95% CI, 14.9%–19.9%), and 3.1% (95% CI, 0.4%–8.4%), respectively, as detailed in **Table 2** and Supplemental Material 3: Figure 4.

Seven studies[11,14,20,23,25,26,30] with 389 patients with severe COVID-19 and 1399 control patients with nonsevere COVID-19 showed a combined WMD of –24.56 (I^2 = 53%; 95% CI, –33.73 to –15.39), indicating that the platelet number was lower in patients with more severe COVID-19 (**Table 2** and Supplemental Material 3: Figure 5). Consistent with these results, a meta-analysis of 8 studies[2,3,11,12,16,21,22,24] including 510 patients with adverse events (including admission to ICU, progression to ARDS, or death) and 1950 patients without adverse events revealed a combined WMD of –22.48 (I^2 = 78%; 95% CI, –40.97 to –3.99; **Table 2** and Supplemental Material 3: Figure 6). Subgroup analysis indicated the platelet count to be even lower in patients who succumbed to the disease (WMD, –49.02; I^2 = 0%; 95% CI, –60.26 to –37.78]); however, no significant difference was observed between patients admitted to the ICU and those who were not (WMD, 0.41; I^2 = 0%; 95% CI, –20.04 to 20.87]; **Table 2** and Supplemental Material 3: Figure 7). Taken together, these results highlight the lower platelet number in patients with either more severe COVID-19 or poor outcomes. These findings are consistent with those reported by Lippi et al,[6] who first conducted a meta-analysis on 1779 patients to investigate the association between TCP and COVID-19 severity.

DOI: 10.1093/labmed/lmaa067

Table 1. General Characteristics of the Included Studies

Study	Region	N	Male (%)	Age (y)	Severity/ Outcome	Platelet Count			Incidence of TCP				NOS
						Overall	SEV/PO	Non SEV/PO	Overall	With PO (%)	Without PO (%)	Cutoff	
Chen, Liu, et al[9]	Wuhan, China	29	72.0%	56 (median, 26–79)	48.3% severe disease	NR	—	—	17.0%	—	—	<125	4
Chen, Zhou, et al[10]	Wuhan, China	99	68.0%	55·5 ± 13.1	23.0% in ICU	213·5 ± 79.1	—	—	12.0%	—	—	<125	6
Guan et al[11]	30PRs[b], China	1099	58.1%	47 (35–58)	15.7% severe disease	168 (132–207)	137 (99–179)	172 (139–212)	36.2%	46.6	35.5	<150	7
Huang, Wang, et al[12]	Wuhan, China	41	73.0%	49 (41–58)	31.7% in ICU	165 (132–263)	196 (165–263)	149 (131–263)	4.9%	8.0	4.0	<100	7
Huang, Tu, et al[13]	Wuhan, China	34	41.2%	56.2 ± 17.1	23.5% in ICU	NR	—	—	26.5%	—	—	NR	5
Liu, Tao, et al[14]	Wuhan, China	78	50.0%	38 (33–57)	14.1% deterioration	169.1 ± 57.3	143.90 ± 64.81	173.20 ± 55.37	NR	—	—	<100	7
Shi et al[15]	Wuhan, China	81	52.0%	49.5 ± 11.0	4% mortality	NR	—	—	0.0%	—	—	<100	6
Wang et al[16]	Wuhan, China	138	54.3%	56 (42–68)	26.1% in ICU	163 (123–191)	142 (119–202)	165 (125–188)	NR	—	—	<125	7
Wu, Liu, et al[17]	Jiangsu, China	80	48.8%	46.1 ± 15.4	3.8% severe disease	155 (116–188)	—	—	13.8%	—	—	<125	6
Yang, Cao, et al[18]	Zhejiang, China	149	54.4%	45.1 ± 13.4	No severe disease	174.5 ± 78.3	—	—	13.4%	—	—	<125	6
Zhou et al[3]	Wuhan, China	191	62.0%	56 (46–67)	28.3% mortality	206 (155–262)	166 (107–229)	220 (168–271)	7.0%	20.0	1.0	<100	8
Xu et al[19]	Zhejiang, China	62	56.0%	41 (32–52)	No severe disease	176 (136–216)	—	—	5.0%	—	—	<100	6
Young et al[20]	Singapore	18	50.0%	47 (median, 31–73)	33.3% SaO_2 ≤92%	159 (116–217)	159 (128–213)	156 (116–217)	NR	—	—	NR	7
Fan et al[21]	Singapore	67	55.2%	42 (35–54)	13.4% in ICU	NR	217 (154–301)	201 (157–263)	0.0%	—	—	<100	7
Wu, Chen, et al[2]	Wuhan, China	201	63.7%	51 (43–60)	26.4% in ICU	180 (137–242)	187 (125–253)	178 (140–240)	18.8%	—	—	<125	8
Chen, Wu, et al[22]	Wuhan, China	274	62.0%	62 (44–70)	41.2% mortality	179 (133–235)	156 (112–219)	198 (160–256)	NR	—	—	<125	7
Mo et al[23]	Wuhan, China	155	55.5%	54 (42–66)	54.8% refractory disease	170 (127–208)	159 (119–202)	179 (146–219)	NR	—	—	<125	7
Tang et al[24]	Wuhan, China	449	59.7%	65.1 ± 12.0	29.8% mortality	215 ± 100	178 ± 92	231 ± 99	21.6%	—	—	<150	7
Qu et al[25]	Guangzhou, China	30	53.3%	50.5 (median, 36–65)	10.0% severe disease	NR	169.7 ± 48.9	192.3 ± 58.1	NR	—	—	NR	6
Wan et al[26]	Chongqing, China	135	53.3%	47 (36-55)	29.6% severe disease	158 (131–230)	147 (118–213)	170 (136–234)	17.0%	30.0	11.6	<125	6
Zhang, Zhang, et al[27]	Wuhan, China	95	55.8%	49 (39–58)	33.7% severe disease	NR	—	—	11.6%	20.0	8.6	<100	7
Liu, Sun, et al[28]	Wuhan, China	383	42.3%	46 (34–61)	12.8% mortality	174 (137–213)	—	—	17.8%	42.9	14.1	<125	8
Yang, Yang, et al[29a]	Wuhan, China	1476	52.6%	NR	16.1% mortality	NR	79 (43 - 129)	203 (155 -257)	20.7%	72.7	10.7	<125	8
Yang, Shi, et al[30]	Shanghai, China	273	49.1%	49.1	26.0% CT scan progression	NR	176.0 ± 6.6	195.0 ± 5.1	NR	—	—	NR	6

Data presented as median (interquartile range), median (range), or mean ± standard deviation. Poor outcomes included a composite of admission to ICU, progression to ARDS, and mortality.

[a] *This study reported the nadir platelet count during hospitalization duration.*

[b] *Provinces (PRs) include all provinces or provincial municipalities of China, except Hong Kong, Macau, and Tibet.*

SEV, severe; PO, poor outcomes; PRs, provinces; NOS, Newcastle-Ottawa Scale; NR, not reported.

Table 2. Results Summary of Pooled Analyses and Subgroup Analysis

Combined Estimation Subgroup Analysis	Number of Studies (Patients)	I^2	Cochran's Q *P* Value	Effect Size
				Proportion (95%CI)
Incidence of TCP	17 (4671)	95%	.02	12.4% (7.9% to 17.7%)
Cutoff <150	2 (1548)	97%	.00	28.7% (15.6% to 43.9%)
Cutoff <125	8 (2552)	43%	.09	17.3% (14.9% to 19.9%)
Cutoff <100	6 (537)	86%	.00	3.1% (0.4% to 8.4%)
				WMD (95%CI)
Severe vs nonsevere	7 (1788)	53%	.05	−24.56 (−33.73 to −15.39)[a]
With PO vs without PO	8 (2460)	78%	.00	−22.48 (−40.97 to −3.99)[a]
Mortality	3 (914)	0%	.67	−49.02 (−60.26 to −37.78)[a]
Admission to ICU	3 (246)	0%	.57	0.41 (−20.04 to 20.87)
ARDS	1 (201)	NA	NA	2.17 (−22.52 to 26.86)
Composite endpoint	1 (1099)	NA	NA	−14.67 (−29.74 to 0.40)
				OR (95%CI)
With TCP vs without TCP	5 (1578)	67%	.02	3.49 (1.57 to 7.78)[a]
Mortality	2 (574)	60%	.11	7.37 (2.08 to 26.14)[a]
Admission to ICU	1 (40)	NA	NA	2.17 (0.12 to 37.64)
Composite endpoint	2 (964)	0%	.46	1.17 (1.04 to 2.80)[a]
Cutoff <150	1 (869)	NA	NA	1.58 (0.93 to 2.70)
Cutoff <125	1 (383)	NA	NA	4.58 (2.40 to 8.72)[a]
Cutoff <100	3 (326)	47%	.15	5.22 (1.30 to 20.93)[a]

[a]*Z test, P <.05. Composite endpoint included admission to ICU, the use of mechanical ventilation, and mortality.*
PO, poor outcomes; NA, not applicable.

To investigate the association of TCP with COVID-19 outcome, 5 studies[3,11,12,27,28] reporting a total of 199 adverse events (65 adverse events with TCP [15.9 per 100 participants] and 134 without TCP [11.5 per 100 participants]) were included. The combined OR of 3.49 (I^2 = 67%; 95% CI, 1.57–7.78) suggests TCP as a potential risk factor for poor outcomes in COVID-19 (**Table 2** and Supplemental Material 3: Figure 8). Furthermore, the subgroup analysis by endpoint events showed that TCP was associated with a 7-fold enhanced risk of mortality in COVID-19 (OR, 7.37; I^2 = 36%; 95% CI, 2.08–26.14). A recent study indicated that platelet count was associated with in-hospital mortality in a dose-dependent manner.[28] Consistent with this finding, we found a stronger association between TCP and poor outcomes of COVID-19 when a lower cutoff TCP value was applied (**Table 2** and Supplemental Material 3: Figure 9).

To some extent, ARDS onset, admission to ICU, and mortality can be considered as sequential events reflective of COVID-19 progression and deterioration.[3] One advantage of the current study was the inclusion of many patients with multiple outcomes, allowing subgroup analysis to investigate the association of TCP with distinct endpoints. A surprising but interesting finding of the subgroup analysis is that TCP (both platelet number decrease and TCP rate) may not be significantly related to ICU admission (**Table 2** and Supplemental Material 3: Figures 7, 10) despite the trend of association between TCP and mortality and the composite endpoint (admission to ICU, use of mechanical ventilation, and mortality). A possible explanation for this result is that TCP tends to reach a significant level in the late clinical stage of COVID-19. This speculation can be supported by a prior study that reported a lower platelet number in patients hospitalized for more than 10 days after symptom onset than in those hospitalized within 10 days of symptom onset.[19] Two recent studies that investigated the dynamic change in platelet count of patients with COVID-19 described a trend of persistent drop in platelet number for nonsurvivors, with a decline to 100 × 10^9/L after approximately 2 weeks after admission.[28,29] These results suggest that TCP is associated with COVID-19 progression and deterioration, highlighting the significance of monitoring platelet count. However, the results of the subgroup analysis may be limited by the small subgroup size and need further validation.

Although the role of TCP in COVID-19 has been well studied, the causal relationship between them is not yet established. Several possible mechanisms may be involved in platelet count fluctuations during the pathophysiological process of this viral disease. COVID-19 is a systemic infection characterized by hyperinflammation and hypercoagulable state in patients with severe disease.[32]

DOI: 10.1093/labmed/lmaa067

Hence, TCP in COVID-19 may be explained by the irreversible consumption of platelets during the execution of procoagulant and immune modulation functions. Viral infection of megakaryocytes can increase their apoptosis and decrease maturation and ploidy.

Although there is no evidence that SARS-CoV-2 can directly infect the bone marrow or hematopoietic stem cells, the virus may modulate platelet production at other stages of development. For example, thrombopoietin (TPO), which is primarily produced in liver parenchymal cells, is a critical regulator of megakaryopoiesis and platelet production. Several reports have suggested that liver injury is a common complication of COVID-19.[33,34] A drop in TPO production after parenchymal liver injury may at least in part explain TCP in COVID-19. Recently, a series of case reports suggested that COVID-19 is associated with the onset or reoccurrence of immune thrombocytopenia (ITP), which is characterized by isolated TCP, without thrombosis tendency and bone marrow abnormal cellularity.[35-38] These findings suggested that the differential diagnosis of TCP in COVID-19 is indispensable; for SARS-CoV-2 infection–induced ITP, accurate diagnosis and specialized treatment are necessary to guarantee the prevention of bleeding episodes. Further investigation of the mechanisms of TCP can provide a more comprehensive understanding of this disease and provide a theoretical basis for clinical treatment.

In conclusion, this systematic review shows that TCP is common in COVID-19, as evident from a weighted incidence of 12.4%, and is associated with COVID-19 severity and outcome. Thus, TCP may serve as a potential biomarker to predict mortality in patients with COVID-19. A limitation of this study is that only 17 of the 24 included studies were available for synthesis for primary outcome, whereas all other subgroup analyses included data from <10 studies. Therefore, the results from subgroup analyses are uncertain and should be regarded with extreme caution. Further, most of the included studies were cross-sectional investigations, and the asymmetrical shape of the funnel plot (Supplemental Material 3: Figure 11) shows evidence of publication bias, suggestive of the high risk of bias involved in our meta-analysis. Because studies with strong quality evidence such as randomized controlled trials and case-control studies are not feasible or available in the context of the COVID-19 pandemic, individual patient data meta-analysis is warranted to address these questions in the future.

Supplementary Data

Supplemental figures and tables can be found in the online version of this article at www.labmedicine.com. **LM**

References

1. Situation Report-173. World Health Organization. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200711-covid-19-sitrep-173.pdf?sfvrsn=949920b4_2. Published July 11, 2020. Accessed July 12, 2020.
2. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;180(7):1–11.
3. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395(10229):1054–1062.
4. Alonso AL, Cox D. Platelet interactions with viruses and parasites. *Platelets.* 2015;26(4):317–323.
5. Assinger A, Schrottmaier WC, Salzmann M, Rayes J. Platelets in sepsis: an update on experimental models and clinical data. *Front Immunol.* July 2019;10:1687.
6. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. *Clin Chim Acta.* July 2020;506:145–148.
7. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ.* July 2009;339:b2535.
8. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res.* 2018;27(6):1785–1805.
9. Chen L, Liu HG, Liu W, et al. Clinical characteristics analysis of 29 cases of 2019 novel coronavirus pneumonia. *Chinese Journal of Tuberculosis and Respiratory Diseases.* 2020;43(0):E005.
10. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020;395(10223):507–513.
11. Guan WJ, Ni ZY, Hu Y, et al.; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* 2020;382(18):1708–1720.
12. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497–506.
13. Huang Y, Tu M, Wang S, et al. Clinical characteristics of laboratory confirmed positive cases of SARS-CoV-2 infection in Wuhan, China: a retrospective single center analysis [published online ahead of print February 27, 2020]. *Travel Med Infect Dis*. 2020. doi: 10.1016/j.tmaid.2020.101606.
14. Liu W, Tao ZW, Wang L, et al. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. *Chin Med J (Engl).* 2020;133(9):1032–1038.
15. Shi H, Han X, Jiang N, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis.* 2020;20(4):425–434.

16. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061–1069.
17. Wu J, Liu J, Zhao X, et al. Clinical characteristics of imported cases of COVID-19 in Jiangsu Province: a multicenter descriptive study. *Clin Infect Dis*. 2020;71(15):706–712.
18. Yang W, Cao Q, Qin L, et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19): a multi-center study in Wenzhou city, Zhejiang, China. *J Infect.* 2020;80(4):388–393.
19. Xu XW, Wu XX, Jiang XG, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *BMJ.* February 2020;368:m606.
20. Young BE, Ong SWX, Kalimuddin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA*. 2020;323(15):1488–1494.
21. Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID-19 infection. *Am J Hematol.* 2020;95(6):E131–E134.
22. Chen T, Wu D, Chen H, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ*. March 2020;368:m1091.
23. Mo P, Xing Y, Xiao Y, et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China [published online ahead of print March 16, 2020]. *Clin Infect Dis*. doi: 10.1093/cid/ciaa270.
24. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost.* 2020;18(5):1094–1099.
25. Qu R, Ling Y, Zhang YH, et al. Platelet-to-lymphocyte ratio is associated with prognosis in patients with coronavirus disease-19 [published online ahead of print March 17, 2020] *J Med Virol.* doi: 10.1002/jmv.25767.
26. Wan S, Xiang Y, Fang W, et al. Clinical features and treatment of COVID-19 patients in northeast Chongqing. *J Med Virol*. 2020;92(7):797–806.
27. Zhang G, Zhang J, Wang B, Zhu X, Wang Q, Qiu S. Analysis of clinical characteristics and laboratory findings of 95 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a retrospective analysis. *Respir Res.* 2020;21(1):74.
28. Liu Y, Sun W, Guo Y, et al. Association between platelet parameters and mortality in coronavirus disease 2019: retrospective cohort study. *Platelets*. 2020;31(4):1–7.
29. Yang X, Yang Q, Wang Y, et al. Thrombocytopenia and its association with mortality in patients with COVID-19. *J Thromb Haemost.* 2020;18(6):1469–1472.
30. Yang Z, Shi J, He Z, et al. Predictors for imaging progression on chest CT from coronavirus disease 2019 (COVID-19) patients. *Aging (Albany NY).* 2020;12(7):6037–6048.
31. Borges do Nascimento IJ, Cacic N, Abdulazeem HM, et al. Novel coronavirus infection (COVID-19) in humans: a scoping review and meta-analysis. *J Clin Med*. 2020;9(4):941.
32. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. *Am J Hematol.* 2020;95(7):834–847.
33. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol.* 2020;5(5):428–430.
34. Lei F, Liu YM, Zhou F, et al. Longitudinal association between markers of liver injury and mortality in COVID-19 in China [published online ahead of print May 2, 2020]. *Hepatology*. doi: 10.1002/hep.31301.
35. Zulfiqar AA, Lorenzo-Villalba N, Hassler P, Andrès E. Immune thrombocytopenic purpura in a patient with Covid-19. *N Engl J Med.* 2020;382(18):e43.
36. Chen W, Yang B, Li Z, et al. Sudden severe thrombocytopenia in a patient in the recovery stage of COVID-19. *Lancet Haematol*. 2020;7(8):e624.
37. Merli M, Ageno W, Sessa F, et al. Recurrence of immune thrombocytopenia at the time of SARS-CoV-2 infection. *Ann Hematol.* 2020;99(8):1951–1952.
38. Murt A, Eskazan AE, Yılmaz U, et al. COVID-19 presenting with immune thrombocytopenia: a case report and review of the literature [published online ahead of print June 4, 2020]. *J Med Virol*. doi: 10.1002/jmv.26138.

Autophagy in Hematological Malignancies: Molecular Aspects in Leukemia and Lymphoma

Hassan Boustani, MSc,[1] Elahe Khodadi, MSc,[2] Minoo Shahidi, PhD[1]*

Laboratory Medicine 2021;52:16-23

DOI: 10.1093/labmed/lmaa027

ABSTRACT

The organization of the hematopoietic system is dependent on hematopoietic stem cells (HSCs) that are capable of self-renewal and multilineage differentiation to produce different blood cell lines. Autophagy has a central role in energy production and metabolism of the cells during starvation, cellular stress adaption, and removing mechanisms for aged or damaged organelles.

The role and importance of autophagy pathways are becoming increasingly recognized in the literature because these pathways can be useful in organizing intracellular circulation, molecular complexes, and organelles to meet the needs of various hematopoietic cells. There is supporting evidence in the literature that autophagy plays an emerging role in the regulation of normal cells and that it also has important features in malignant hematopoiesis. Understanding the molecular details of the autophagy pathway can provide novel methods for more effective treatment of patients with leukemia. Overall, our review will emphasize the role of autophagy and its different aspects in hematological malignant neoplasms.

Keywords: autophagy, hematological malignant neoplasms, hematopoiesis, molecular, leukemia, lymphoma

Abbreviations

ROS, reactive oxygen species; ATG, autophagy-related genes; HSPCs, high self-renewing precursor cells; LSCs, leukemic stem cells; MRD, minimal residual disease; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; miR, microRNA; FLT3, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; NPM, nucleophosmin 1; PML, promyelocytic leukemia; Akt, serine/threonine-protein kinase; LICs, leukemia-initiating cells; MLL-ENL, mixed lineage leukemia–eleven nineteen lysine-rich leukemia; ATG7, autophagy-related 7; ATG5, autophagy-related 5; AML1-ETO, acute myelogenous leukemia–1 eight-twenty-one oncoprotein; MLL, mixed-lineage leukemia; BM, bone marrow; HLX, H2.0-like homeobox transcription factor; AMPK, AMP-activated protein kinase; CML, chronic myeloid leukemia; BCR-ABL, breakpoint cluster region protein–Abelson murine leukemia; PIK-III, PtIns3P class III inhibitor; CCNG2, Cyclin-G2; MDS, myelodysplastic syndrome; MDS-RS, myelodysplastic syndrome with ring sideroblasts; ATG2B, autophagy-related protein 2 homolog B; GSKIP, GSK3B-interacting protein; ALL, acute lymphoid leukemia; B-ALL, acute lymphoid leukemia in B-cell precursors; T-ALL, acute lymphoid leukemia in thymocytes; BECN1, Beclin-1; pre-B ALL, pre-B acute lymphoblastic leukemia; CLL, chronic lymphoid leukemia; SLL, small lymphocytic lymphoma; B-CLL, B-cell chronic lymphocytic leukemia; TLR9, TolI-like receptor 9; IgM, immunoglobulin M; PI3K, phosphoinositide 3-kinase; PIK3R4, phosphoinositide-3-kinase regulatory subunit 4; DAPK1, death-associated protein kinase 1; CEBP-ATF6, CCAAT-enhancer-binding proteins–activating transcription factor 6; SLAMF1, signaling lymphocytic activation molecule family member 1; DLBCL, diffused large-cell B lymphoma; B-NHL, B–non-Hodgkin lymphoma; FL, follicular lymphoma; WHO, World Health Organization; BCL, B-cell lymphoma; CUL4B, Cullin-4B; JNK, c-Jun N-terminal kinases; MCL, mantle cell lymphoma; TG2, tissue transglutaminase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PLSCR1, phospholipid scramblase 1; hH, hedgehog; CXCR-4, C-X-C chemokine receptor type 4; BL, Burkitt lymphoma; MM, multiple myeloma; LC3, light chain 3; MDSCs, myeloid-derived suppressor cells; MALAT-1, metastasis-associated lung adenocarcinoma transcript–1; HMGB1, high mobility group box 1; mTORC, mammalian target of rapamycin complex; ALCL, anaplastic large-cell lymphoma; siRNA, small interfering RNA; HIF-1, hypoxia-inducible factor 1; HSCs, hematopoietic stem cells; FL, follicular lymphoma

[1]Department of Hematology and Blood Banking, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran, [2]Thalassemia & Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

*To whom correspondence should be addressed.
Shahidi.m@iums.ac.ir

Autophagy is a vesicular pathway in which the cellular components are moved to autophagosome vesicle and then will degenerate in lysosomes.[1] Autophagy is created in different conditions, such as nutrient- and energy-limiting conditions, endoplasmic reticulum stress, reactive oxygen species (ROS), hormonal imbalance, and exposure to microorganisms.[2] Autophagy plays an important role in cellular renewal of quiescent and differentiated cells, physiological processes, cell survival, and immune responses. This process is defective in inflammatory conditions, infections, cancer, neurodegenerative disorders, and aging.[3]

The results of studies[4] on yeast have revealed autophagy-related genes (ATG) that produce autophagosomes by a multistep process. The role of autophagy is complicated in cancer, depending on the stage, type, and driving oncogene in tumor cells. Study results[5,6] have shown the role of autophagy in tumor formation and metastasis in protection against apoptosis and support of cell survival. Also, the mechanisms involved in the therapy-resistance of tumor cells may occur due to autophagic response to therapy in tumor cells.

Hematopoiesis is a fully coordinated process in which hematopoietic stem pools and high self-renewing precursor cells (HSPCs) can form lymphoid and myeloid lineages. The HSPC pool decreases with aging, which results in a small number of HSPC clones that maintain hematopoiesis; with the decrease in blood-cell diversity, a phenomenon called clonal hematopoiesis occurs. Clonal proliferation in HSPCs that have a specific genetic mutation results in an increased risk of developing blood-based malignant neoplasms (**Figure 1**).[7]

Stem cell proliferation and differentiation in many tissues, including the hematopoietic system, is associated with dysregulation in malignant neoplasms. Leukemia cells have genetic and epigenetic defects that make them genetically distinct from normal blood cells. Hematopoietic cells are transformed at the stem cell stage and move towards the leukemic form. The initiation of leukemia, its progression, its resistance to treatment, and the recurrence of the disease are due to the proliferation of stem cells that are inherently resistant to conventional chemotherapy treatments.[8] Because leukemic stem cells (LSCs) also maintain minimal residual disease (MRD) and recur after treatment, a targeted approach is needed to eradicate them and provide sustainable treatment to patients.[9]

Early-onset autophagy increases the occurrence of hematological malignant neoplasms and increases the protumoral response that occurs in tumor growth stages by autophagy and in response to treatment modalities.[10] There is some evidence[11] that modulation of autophagy pathways can be a useful and effective therapeutic approach in patients with leukemia. Therefore, in this review, we discuss the autophagy pathway and its different molecular aspects in the development of hematological malignant neoplasms, including myeloid and lymphoid leukemia and lymphoma.

Autophagy in Myeloid Malignant Neoplasms

Acute Myeloid Leukemia (AML)

AML, the most common type of leukemia in older adults (80%), is characterized by arrest of myeloid differentiation and inappropriate proliferation and survival in leukemic blast cells associated with high clinical heterogeneity among individuals. Evidences[12,13] suggest that primary blasts in AML have lower autophagy gene levels, compared with nonleukemic cells or AML differentiation cells. Similarly, increased expression of autophagy receptor SQSMT1/p62 has been observed[14] during neutrophil differentiation in acute promyelocytic leukemia (APL) cells.

It has also been reported[14] that microRNA (miR)–17, -20, -93, and -106, which target SQSMT1/p62, are higher in mouse and human blast cells than in neutrophils. Increased expression of SQSMT1/p62 is thought[15,16] to prevent ubiquitinated protein accumulation. During the final differentiation of APL cells, it acts as a prosurvival cellular mechanism. Recent evidence[17] also suggests that SQSMT1/p62 is essential for cell growth and maintaining mitochondrial integrity. In fact, defects in SQSMT1/p62 impair the progression of myeloid leukemia and mitophagy in this type of malignant neoplasm.

Numerous articles in the literature support the idea that the role of autophagy in the progression of leukemia varies depending on the type of oncogene that can affect disease progression. The RET proto-oncogene is a tyrosine kinase that has recently been identified[18] as a key kinase in AML. Activated RET pathways lead to decreased autophagy and stability of leukemia-causing factors, such as mutant FMS-like tyrosine kinase 3 (FLT3). Also, RET inhibition leads to

Lab Medicine 2021;52;16–23 17
DOI: 10.1093/labmed/lmaa027

decreased FLT3 through autophagy. However, proteasome inhibitors increase FLT3 internal tandem duplication (FLT3/ITD) degeneration through autophagy.[19]

In contrast, inhibition of the FLT3-ITD mutation in AML cells, by impairing autophagy-dependent proliferation in vitro and in vivo, enhances a high level of basal autophagy. This FLT3-ITD–dependent autophagy depends on the extent of ATF4 transcription.[20] In AML cells mutated with nucleophosmin 1 (NPM), promyelocytic leukemia (PML) protein remains stable in the cytoplasm. PML insertion in the cytoplasm leads to serine/threonine-protein kinases (Akt) phosphorylation, which subsequently results in prosurvival autophagy.[21]

In studies of MLL-ENL mouse models, inhibition of autophagy by ATG7 deletion leads to decreased leukemia-initiating cells (LICs) and stronger survival in leukemic mice. These conditions led to an increase in ROS production, along with an increase in mitochondrial activity and cell death. Accordingly, blasts of this mouse model were reduced in peripheral blood due to increased apoptosis.[22]

Study results have shown that in the mixed lineage leukemia–eleven nineteen lysine-rich leukemia (MLL-ENL) mouse model, inhibition of autophagy by removing autophagy-related 7 (ATG7) or autophagy-related 5 (ATG5) results in more aggressive leukemia in vivo. In addition, these results showed that MLL-ENL cells with decreased autophagy activity lead to abnormal activity in mitochondria—specifically, proliferation and transformation.

In the acute myelogenous leukemia–1 eight-twenty-one oncoprotein (AML1-ETO) AML mouse model, autophagy inhibited LIC proliferation.[23] Mixed-lineage leukemia (MLL-Af9, or MA9) AML cells exhibit more autophagic activity than normal bone marrow (BM). However, degradation of Rb1cc1 or ATG5 does not affect the growth or survival of MA9-AML cells, in vitro and in vivo.[24] Study results[25] have shown that autophagy activity is required for malignancy but not required for maintenance of malignancy in MLL-Af9 AML cells.

The H2.0-like homeobox transcription factor (HLX), which shows increased expression in AML, is another factor involved in the regulation of hematopoietic differentiation. Increased expression of HLX has been reported to induce 5′ AMP-activated protein kinase (AMPK) activation and survival of AML cells through autophagy.[26] Overall, AML is a heterogeneous disease, and so autophagy acts as a tumor promoter and suppressor, depending on the AML subtype.

Chronic Myeloid Leukemia (CML)

CML accounts for 15% of all types of leukemia in adults, with displacement of t(22;9)(q34;q11) and expression of the breakpoint cluster region protein–Abelson murine leukemia viral oncogene homolog 1 (BCR-ABL) fusion protein, which has excessive tyrosine kinase activity. Study results[26] have shown that BCR-ABL1 induces MAPK15-dependent autophagy and causes cell transformation. However, other study results[27] have shown that BCR-ABL inhibits ATF5-dependent autophagy only in transformed cells. Lys05, the second generation of autophagy inhibitors, results in quiescence of CML stem cells and reduces the maturation of these cells. Also, Lys05 or PIK-III (PtIns3P class III inhibitor), when selectively combined with a tyrosine kinase inhibitor, reduced the number of primary CML LSCs. This finding suggests that this combination drug is associated with the destruction of stem cells in patients with CML.[27]

By using a certain type of cell culture and keeping the cells primarily at low oxygen concentration and subsequently exposed to oxygen, KML5 cells need autophagy only for commitment and not for proliferation.[28] Also, BIM1 expression as a transcription factor promotes CML progression to acute stages.[29] In fact, BIM1 inhibition increases Cyclin-G2 (CCNG2) expression and decreases tumor suppressor response in autophagy.

Myelodysplastic Syndrome (MDS)

MDS comprises a heterogeneous class of blood disorders.[30,31] It is divided into 4 main subgroups: MDS with single-lineage dysplasia, MDS with ring sideroblasts (MDS-RS), MDS with excess blasts, and MDS with isolated del(5q).

Numerous studies are underway to help researchers understand the effect of autophagy on the development and development of MDS. Increased expression of autophagy-related protein 2 homolog B (ATG2B) and GSK3B-interacting protein (GSKIP) caused changes in germline copy number in several myeloid malignant neoplasms.[32] However, study results[33] have shown that patients with MDS who harbor U2AF35 (S35F) mutation show an increase in pre-mRNA levels in ATG7 and have a distal cleft at the polyadenylation site, which reduce ATG7 expression and autophagy activity in cells. Nuclear red blood cells in patients with high-risk MDS have lower LC3B levels and greater mitochondrial deficiency; also, LC3B levels are

correlated with hemoglobin levels in these patients.[34] In addition, ATG3 expression is lower in patients with MDS than in healthy control individuals, and overexpression of ATG3 in the SKM-1 MDS cell line increases caspase-dependent autophagy activity and cell death.[35]

Autophagy in Lymphoid Malignancies

B- and T-lymphoblastic Leukemia/Lymphoma

Acute lymphoid leukemia (ALL) is the most common blood malignant neoplasm in children and accounts for 20% of adult leukemia cases.[36] This heterogeneous disorder involves different genomic changes, such as changes in the number and structure of chromosomes, and changes in the number of copies and mutations of DNA. This malignant neoplasm mainly occurs in B-cell precursors (B-ALL; 80%) and 20% in thymocytes (T-ALL). The results of a study[36] of 1 splice variant in Beclin-1 (BECN1) in the ALL 697 cell line with deletion in exon 11 have shown a decrease in autophagy induction in this malignant neoplasm.

We note that the ALL 697 cell line in the vicinity of Bafilomycin A1 decreased the graft in NOD/SCID mice. However, chromosomal translocation t (1;19) in pre-B acute lymphoblastic leukemia (pre-B ALL) in children induces autophagy in the face of rapamycin, leading to destruction of POLD1 DNA and RNA pol, and inhibits cell growth.[37] Also, using Torin-2 in pre-B ALL cells inhibits mTOR activity, enhances autophagy, and inhibits cell growth, which points to a tumor-suppressor role in autophagy.[38]

The role of autophagy in lymphoid malignant neoplasms is still controversial and may be observed in a specific subgroup. Further studies are needed to better understand the role of this recycling mechanism in leukemogenesis in the lymphoid lineage.

Chronic Lymphoid Leukemia (CLL)/Small Lymphocytic Lymphoma (SLL)

B-cell chronic lymphocytic leukemia (B-CLL)/SLL occurs predominantly in older adults and is characterized by inherent defects in cell death. This malignancy progresses slowly and shows clinical symptoms in afflicted patients. Study results[39] have shown that RNA inhibition of autophagy by targeting key autophagy genes in patients with CLL reduces cell survival.

Autophagy is also required for Toll-like receptor 9 (TLR9)–dependent secretion of immunoglobulin M (IgM) in a CLL-positive mouse model. High expression of phosphoinositide 3-kinase (PI3K), phosphoinositide-3-kinase regulatory subunit 4 (PIK3R4), and BECN1 is also associated with poor clinical outcome.[40]

Death-associated protein kinase 1 (DAPK1) is an autophagy-dependent gene 114; in rare cases of CLL, mutation in this gene increases HOXB7 binding to its promoter.[41] The loss of DAPK1 expression in CLL is due to a defect in the CCAAT-enhancer-binding proteins–activating transcription factor 6 (CEBP-ATF6) pathway function. Inhibition of DAPK1 reduces autophagy and increases the growth and proliferation of CLL cells.[42]

The dual role of autophagy in cancer has been proven in various studies. Signaling lymphocytic activation molecule family member 1 (SLAMF1) expression is associated with a favorable prognosis in patients with CLL and disappears in its invasive form.[43] Study results[43] have shown that SLAMF1 activates autophagy by stabilizing the BECN1-VPS34 complex; cells without SLAMF1 are less sensitive to autophagy-induced treatment.

Diffused Large Cell B Lymphoma (DLBCL)

DLBCL accounts for approximately 33% of B–non-Hodgkin lymphoma (B-NHL) and is the most common subgroup. DLBCL is an invasive lymphoma, de novo or the result of clinical progression of less-invasive B-NHL types (such as follicular lymphoma [FL] and CLL).

The 2016 World Health Organization (WHO) classification[44] introduced a new category of lymphomas, described as high-grade B-cell lymphoma with translocations related to MYC and B-cell lymphoma (BCL)–2 or BCL-6. BCL-2 directly inhibits autophagy by binding to BECN1; patients with decreased levels of BCL-2 show increased expression of BECN1, with favorable clinical outcomes.[45,46] Study results[47,48] have shown that in +/– BECN1 mice, the incidence of cancers such as lung, liver cancer, or B-cell lymphoma is higher. Other study results[49] have shown that inhibition of autophagic responses to DLBCL induced by BCL-6 may lead to lymphomagenesis. Also, a link between Cullin-4B (CUL4B) and autophagy and DLBCL progress

DOI: 10.1093/labmed/lmaa027

has been observed.[50] CUL4B regulates autophagy through c-Jun N-terminal kinases (JNK signaling); inhibition of cell proliferation by deletion mutation in CUL4B may inhibit autophagy-mediated cell survival. This finding highlights the complexity of autophagy pathways in the DLBCL.

Mantle Cell Lymphoma (MCL)

MCL is an invasive disease in older adults that accounts for approximately 5% of B-NHL. It is associated with high expression of tissue transglutaminase (TG2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).[51] Decreased expression of TG2 causes proliferation of cells in this disease. Also, TG2 regulates autophagy, and inhibition of autophagy by the ATG5 gene-silencing results in TG2 loss of expression. In addition, ATG5-free cells reproduce less slowly than cells with autophagy.

Phospholipid scramblase 1 (PLSCR1) is a proapoptotic gene that, through 9-cis-retinoic and interferon-α, decreases its expression in MCL cells.[52,53] Also, PLSCR1 inhibits autophagy and reduces the survival of malignant cells in MCL.[54] In contrast, the hedgehog (hH) pathway in MCL cells increases the penetration of malignant cells into the BM. Inhibition of the hH pathway using LDE225 increased C-X-C chemokine receptor type 4 (CXCR-4) expression and, consequently, ROS increased autophagy activity and cell survival.[55]

Burkitt Lymphoma (BL)

BL is an invasive disease in immature B cells that is known by the rearrangement of the *MYC* gene, with early B cell markers testing positive for the disease and the involved cells having a high mitosis rate. BL is a rare disease that accounts for 1% to 2% of B-NHL cases in adults and 30% of pediatric lymphoma cases. The findings of a study on e-myc transgenic mice[56] have shown that dysfunction of lysosomes using chloroquine has prevented lymphomagenesis, suggesting the association of lymphomagenesis with autophagy in these patients.

Multiple Myeloma (MM)

MM is a plasma B-cell cancer that invades the BM, is associated with high genomic and phenotypic diversity, and is observed in elderly patients.[57] Intron 6 of ATG5 on chromosome 6q21 is a risk loci associated with a high probability of developing MM.[58,59] High expression of BECN1 or microtubule-associated protein 1A/1B-light chain 3 (LC3) is also associated with favorable clinical outcomes.[60] This finding suggests that autophagic dysfunction is crucial to the progression of MM disease.

However, findings from other studies indicate that activation of autophagy is essential for MM survival. A recent publication[61] reports that the role of myeloid-derived suppressor cells (MDSCs) in enhancing survival and proliferation of MM cells by activating the AMPK pathway has been demonstrated and that the prosurvival effect on AMPK may be due to autophagy induction. It has also been shown[62] in BM cells of patients with MM that overexpression of long noncoding RNA metastasis-associated lung adenocarcinoma transcript–1 (MALAT-1) and high mobility group box 1 (HMGB1) results in autophagy and survival.

CHE-1 protein, which is antiapoptotic, interacts with RNA polymerase II and regulates its expression. Expression of this protein is associated with MM progression and is essential for cell growth and survival. CHE-1 is phosphorylated by cellular stress and, by binding to the Redd1 and Deptor promoters, increases their expression and thereby decreases mammalian target of rapamycin complex (mTORC) activity. Expression of CHE-1 induces autophagy activity by induction of mTORC1 and mTORC2, indicating its association with autophagy in MM.[63] The results of several studies, such as Hoang et al,[64] have shown that inhibition of autophagy decreases cell survival in MM. However, other study results[65] point to the role of cell death and its association with autophagy in MM cells.

Anaplastic Large Cell Lymphoma (ALCL)

ALCL accounts for 1% to 3% of adult T-NHL lymphoma and 15% of pediatric lymphoma.[66] The role of autophagy in the development of ALCL has not been determined, to our knowledge. The results of a study[67] using derived cell lines have shown that inhibition of autophagy by small interfering RNA (siRNA) binding to ATG7 or chloroquine has no effect on cell viability.

FL

FL accounts for approximately 20% of B-NHL and is the second most common form of lymphoma worldwide.[68,69] Due to the role of autophagy in the regulation of B-cells, study results[70] have shown that patients with FL had a significant decrease in SQSMT1/p62 and LC3 levels, compared with reactive B-cells; this finding indicates increased autophagy activity in FL. The impairment of autophagy regulation in FL is not associated with overexpression

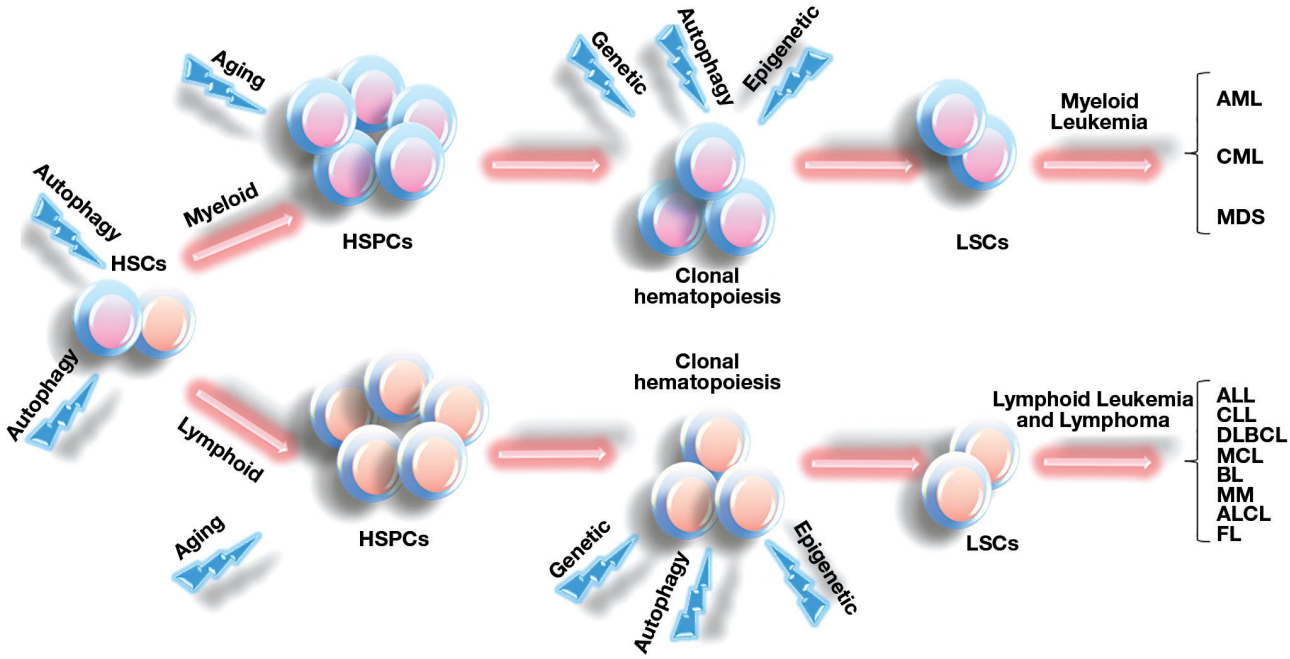


Figure 1

The role of autophagy in normal and malignant cells. Normal high self-renewing precursor cells (HSPCs) can form lymphoid and myeloid lineages by autophagy. Aging results in a decrease and in clonal hematopoiesis of HSPCs. Genetic, epigenetic, and autophagy defects make leukemic stem cells (LSCs) in lymphoid and myeloid cells, which result in myeloid and lymphoid leukemias and lymphomas. HSCs indicates hematopoietic stem cells; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoid leukemia; CLL, chronic lymphoid leukemia; DLBCL, diffused large-cell B lymphoma; MCL, mantle cell lymphoma; BL, Burkitt lymphoma; MM, multiple myeloma; ALCL, anaplastic large-cell lymphoma; FL, follicular lymphoma.

of BCL-2. FL is associated with overexpression of LC3A and overexpression of hypoxia-inducible factor 1 (HIF-1), indicating its association with hypoxia, and has autophagic activity in FL.[71]

Conclusions

Although the role of autophagy in stem-cell transformation and hematological malignant neoplasms has been investigated in many studies, its role in treatment strategies for these disorders is controversial. Also, autophagy modulation has been reported[59] to play a role in outcomes for hematopoietic malignant neoplasms. Understanding the exact molecular mechanism of autophagy in normal and malignant hematopoiesis can help to provide better treatment strategies for patients. Further, each autophagy pathway in every lineage has a specific characteristic, so targeting theses pathways can result in specific strategies for each type of malignant neoplasms. However, further studies are needed to answer remaining questions in this field. LM

References

1. Klionsky DJ, Codogno P. The mechanism and physiological function of macroautophagy. *J Innate Immun.* 2013;5(5):427–433.
2. Kroemer G, Mariño G, Levine B. Autophagy and the integrated stress response. *Mol Cell.* 2010;40(2):280–293.
3. Choi AMK, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med.* 2013;368(7):651–662.
4. Mizushima N, Yoshimori T, Ohsumi Y,. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol*. 2010;27:107–132.
5. Strohecker AM, White E. Autophagy promotes BrafV600E-driven lung tumorigenesis by preserving mitochondrial metabolism. *Autophagy.* 2014;10(2):384–385.
6. Guo JY, White E. Autophagy is required for mitochondrial function, lipid metabolism, growth, and fate of KRAS(G12D)-driven lung tumors. *Autophagy.* 2013;9(10):1636–1638.

DOI: 10.1093/labmed/lmaa027

7. Haybar H, Shahrabi S, Ghanavat M, Khodadi E. Clonal hematopoiesis: genes and underlying mechanisms in cardiovascular disease development. *J Cell Physiol.* 2019;234(6):8396–8401.
8. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med.* 1997;3(7):730–737.
9. Koschade SE, Brandts CH. Selective autophagy in normal and malignant hematopoiesis. *J Mol Biol.* 2020;432(1):261–282.
10. Salio M, Puleston DJ, Mathan TSM, et al. Essential role for autophagy during invariant NKT cell development. *Proc Natl Acad Sci U S A.* 2014;111(52):E5678–E5687.
11. Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat Rev Cancer.* 2008;8(1):11–23.
12. Jin J, Britschgi A, Schläfli AM, et al. Low autophagy (ATG) gene expression is associated with an immature AML blast cell phenotype and can be restored during AML differentiation therapy. *Oxid Med Cell Longev.* 2018;2018:1482795.
13. Watson AS, Riffelmacher T, Stranks A, et al. Autophagy limits proliferation and glycolytic metabolism in acute myeloid leukemia. *Cell Death Dis*. 2015;1:15008.
14. Meenhuis A, van Veelen PA, de Looper H, et al. MiR-17/20/93/106 promote hematopoietic cell expansion by targeting sequestosome 1-regulated pathways in mice. *Blood.* 2011;118(4):916–925.
15. Trocoli A, Bensadoun P, Richard E, et al. p62/SQSTM1 upregulation constitutes a survival mechanism that occurs during granulocytic differentiation of acute myeloid leukemia cells. *Cell Death Differ.* 2014;21(12):1852–1861.
16. Ségal-Bendirdjian E, Tschan MP, Reiffers J, Djavaheri-Mergny M. Pro-survival role of p62 during granulocytic differentiation of acute myeloid leukemia cells. *Mol Cell Oncol.* 2014;1(4):e970066.
17. Nguyen TD, Shaid S, Vakhrusheva O, et al. Loss of the selective autophagy receptor p62 impairs murine myeloid leukemia progression and mitophagy. *Blood.* 2019;133(2):168–179.
18. Rudat S, Pfaus A, Cheng YY, et al. RET-mediated autophagy suppression as targetable co-dependence in acute myeloid leukemia. *Leukemia.* 2018;32(10):2189–2202.
19. Larrue C, Saland E, Boutzen H, et al. Proteasome inhibitors induce FLT3-ITD degradation through autophagy in AML cells. *Blood.* 2016;127(7):882–892.
20. Heydt Q, Larrue C, Saland E, et al. Oncogenic FLT3-ITD supports autophagy via ATF4 in acute myeloid leukemia. *Oncogene.* 2018;37(6):787–797.
21. Zou Q, Tan S, Yang Z, et al. NPM1 mutant mediated PML delocalization and stabilization enhances autophagy and cell survival in leukemic cells. *Theranostics.* 2017;7(8):2289–2304.
22. Sumitomo Y, Koya J, Nakazaki K, et al. Cytoprotective autophagy maintains leukemia-initiating cells in murine myeloid leukemia. *Blood.* 2016;128(12):1614–1624.
23. Man N, Tan Y, Sun X-J, et al. Caspase-3 controls AML1-ETO–driven leukemogenesis via autophagy modulation in a ULK1-dependent manner. *Blood.* 2017;129(20):2782–2792.
24. Chen X, Clark J, Wunderlich M, et al. Autophagy is dispensable for *Kmt2a/Mll-Mllt3/Af9* AML maintenance and anti-leukemic effect of chloroquine. *Autophagy.* 2017;13(5):955–966.
25. Liu Q, Chen L, Atkinson JM, Claxton DF, Wang H-G. Atg5-dependent autophagy contributes to the development of acute myeloid leukemia in an MLL-AF9-driven mouse model. *Cell Death Dis.* 2016;7(9):e2361.
26. Piragyte I, Clapes T, Polyzou A, et al. A metabolic interplay coordinated by HLX regulates myeloid differentiation and AML through partly overlapping pathways. *Nat Commun.* 2018;9(1):3090.
27. Baquero P, Dawson A, Mukhopadhyay A, et al. Targeting quiescent leukemic stem cells using second generation autophagy inhibitors. *Leukemia.* 2019;33(4):981–994.
28. Ianniciello A, Dumas P-Y, Drullion C, et al. Chronic myeloid leukemia progenitor cells require autophagy when leaving hypoxia-induced quiescence. *Oncotarget.* 2017;8(57):96984–96992.
29. Mourgues L, Imbert V, Nebout M, et al. The BMI1 polycomb protein represses cyclin G2-induced autophagy to support proliferation in chronic myeloid leukemia cells. *Leukemia.* 2015;29(10):1993–2002.
30. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol.* 1982;51(2):189–199.
31. Cogle CR, Saki N, Khodadi E, Li J, Shahjahani M, Azizidoost S. Bone marrow niche in the myelodysplastic syndromes. *Leuk Res.* 2015;39(10):1020–1027.
32. Saliba J, Saint-Martin C, Di Stefano A, et al. Germline duplication of ATG2B and GSKIP predisposes to familial myeloid malignancies. *Nat Genet.* 2015;47(10):1131–1140.
33. Park SM, Ou J, Chamberlain L, et al. U2AF35(S34F) promotes transformation by directing aberrant *ATG7* pre-mRNA 3′ end formation. *Mol Cell.* 2016;62(4):479–490.
34. Jiang H, Yang L, Guo L, Cui N, Zhang G, Liu C, et al. Impaired mitophagy of nucleated erythroid cells leads to anemia in patients with myelodysplastic syndromes. *Oxid Med Cell Longev* 2018;2018;6328051.
35. Zhuang L, Ma Y, Wang Q, et al. Atg3 Overexpression enhances bortezomib-induced cell death in SKM-1 cell. *PLoS One.* 2016;11(7):e0158761.
36. Niu Y-N, Liu Q-Q, Zhang S-P, et al. Alternative messenger RNA splicing of autophagic gene Beclin 1 in human B-cell acute lymphoblastic leukemia cells. *Asian Pac J Cancer Prev.* 2014;15(5):2153–2158.
37. Wang Z, Xu F, Yuan N, et al. Rapamycin inhibits pre-B acute lymphoblastic leukemia cells by downregulating DNA and RNA polymerases. *Leuk Res.* 2014;38(8):940–947.
38. Simioni C, Cani A, Martelli AM, et al. Activity of the novel mTOR inhibitor Torin-2 in B-precursor acute lymphoblastic leukemia and its therapeutic potential to prevent Akt reactivation. *Oncotarget.* 2014;5(20):10034–10047.
39. El-Khoury V, Pierson S, Szwarcbart E, et al. Disruption of autophagy by the histone deacetylase inhibitor MGCD0103 and its therapeutic implication in B-cell chronic lymphocytic leukemia. *Leukemia.* 2014;28(8):1636–1646.
40. Kristensen L, Kristensen T, Abildgaard N, et al. High expression of PI3K core complex genes is associated with poor prognosis in chronic lymphocytic leukemia. *Leuk Res.* 2015;39(6):555–560.
41. Raval A, Tanner SM, Byrd JC, et al. Downregulation of *death-associated protein kinase 1 (DAPK1)* in chronic lymphocytic leukemia. *Cell.* 2007;129(5):879–890.
42. Gade P, Kimball AS, DiNardo AC, et al. Death-associated protein kinase-1 expression and autophagy in chronic lymphocytic leukemia are dependent on activating transcription factor-6 and CCAAT/enhancer-binding protein-β. *J Biol Chem.* 2016;291(42):22030–22042.
43. Bologna C, Buonincontri R, Serra S, et al. SLAMF1 regulation of chemotaxis and autophagy determines CLL patient response. *J Clin Invest.* 2016;126(1):181–194.
44. Friedberg JW. How I treat double-hit lymphoma. *Blood.* 2017;130(5):590–596.
45. Nicotra G, Mercalli F, Peracchio C, et al. Autophagy-active beclin-1 correlates with favourable clinical outcome in non-Hodgkin lymphomas. *Mod Pathol.* 2010;23(7):937–950.
46. Huang J-J, Zhu Y-J, Lin T-Y, Jiang W-Q, Huang H-Q, Li Z-M. Beclin 1 expression predicts favorable clinical outcome in patients with diffuse large B-cell lymphoma treated with R-CHOP. *Hum Pathol.* 2011;42(10):1459–1466.
47. Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci U S A.* 2003;100(25):15077–15082.

48. Qu X, Yu J, Bhagat G, et al. Promotion of tumorigenesis by heterozygous disruption of the *beclin 1* autophagy gene. *J Clin Invest.* 2003;112(12):1809–1820.
49. Bertolo C, Roa S, Sagardoy A, et al. *LITAF*, a BCL6 target gene, regulates autophagy in mature B-cell lymphomas. *Br J Haematol.* 2013;162(5):621–630.
50. Li Y, Zhou X, Zhang Y, et al. CUL4B regulates autophagy via JNK signaling in diffuse large B-cell lymphoma. *Cell Cycle.* 2019;18(4):379–394.
51. Zhang H, Chen Z, Miranda RN, Medeiros LJ, McCarty N. TG2 and NF-κB signaling coordinates the survival of mantle cell lymphoma cells via IL6-mediated autophagy. *Cancer Res.* 2016;76(21):6410–6423.
52. Zhou Q, Zhao J, Al-Zoghaibi F, et al. Transcriptional control of the human plasma membrane phospholipid scramblase 1 gene is mediated by interferon-alpha. *Blood.* 2000;95(8):2593–2599.
53. Zhao K-W, Li X, Zhao Q, et al. Protein kinase Cδ mediates retinoic acid and phorbol myristate acetate-induced phospholipid scramblase 1 gene expression: its role in leukemic cell differentiation. *Blood.* 2004;104(12):3731–3738.
54. Mastorci K, Montico B, Faè DA, et al. Phospholipid scramblase 1 as a critical node at the crossroad between autophagy and apoptosis in mantle cell lymphoma. *Oncotarget.* 2016;7(27):41913–41928.
55. Zhang H, Chen Z, Neelapu SS, Romaguera J, McCarty N. Hedgehog inhibitors selectively target cell migration and adhesion of mantle cell lymphoma in bone marrow microenvironment. *Oncotarget.* 2016;7(12):14350–14365.
56. Maclean KH, Dorsey FC, Cleveland JL, Kastan MB. Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis. *J Clin Invest.* 2008;118(1):79–88.
57. Kuehl WM, Bergsagel PL. Molecular pathogenesis of multiple myeloma and its premalignant precursor. *J Clin Invest.* 2012;122(10):3456–3463.
58. Mitchell JS, Li N, Weinhold N, et al. Genome-wide association study identifies multiple susceptibility loci for multiple myeloma. *Nat Commun.* 2016;7:12050.
59. Went M, Sud A, Försti A, et al; PRACTICAL consortium. Identification of multiple risk loci and regulatory mechanisms influencing susceptibility to multiple myeloma. *Nat Commun.* 2018;9(1):3707.
60. Jung G, Roh J, Lee H, et al. Autophagic markers BECLIN 1 and LC3 are associated with prognosis of multiple myeloma. *Acta Haematol.* 2015;134(1):17–24.
61. De Veirman K, Menu E, Maes K, et al. Myeloid-derived suppressor cells induce multiple myeloma cell survival by activating the AMPK pathway. *Cancer Lett.* 2019;442:233–241.
62. Gao D, Lv A-E, Li H-P, Han D-H, Zhang Y-P. LncRNA MALAT-1 elevates HMGB1 to promote autophagy resulting in inhibition of tumor cell apoptosis in multiple myeloma. *J Cell Biochem.* 2017;118(10):3341–3348.
63. Desantis A, Bruno T, Catena V, et al. Che-1-induced inhibition of mTOR pathway enables stress-induced autophagy. *EMBO J.* 2015;34(9):1214–1230.
64. Hoang B, Benavides A, Shi Y, Frost P, Lichtenstein A. Effect of autophagy on multiple myeloma cell viability. *Mol Cancer Ther.* 2009;8(7):1974–1984.
65. Lamy L, Ngo VN, Emre NCT, et al. Control of autophagic cell death by caspase-10 in multiple myeloma. *Cancer Cell.* 2013;23(4):435–449.
66. Turner SD, Lamant L, Kenner L, Brugières L. Anaplastic large cell lymphoma in paediatric and young adult patients. *Br J Haematol.* 2016;173(4):560–572.
67. Mitou G, Frentzel J, Desquesnes A, et al. Targeting autophagy enhances the anti-tumoral action of crizotinib in ALK-positive anaplastic large cell lymphoma. *Oncotarget.* 2015;6(30):30149–30164.
68. Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. *J Clin Invest.* 2012;122(10):3424–3431.
69. Gravelle P, Do C, Franchet C, et al. Impaired functional responses in follicular lymphoma $CD8^{+}TIM\text{-}3^{+}$ T lymphocytes following TCR engagement. *Oncoimmunology.* 2016;5(10):e1224044.
70. McCarthy A, Marzec J, Clear A, et al. Dysregulation of autophagy in human follicular lymphoma is independent of overexpression of BCL-2. *Oncotarget.* 2014;5(22):11653–11668.
71. Giatromanolaki A, Koukourakis MI, Pouliliou S, et al. Overexpression of LC3A autophagy protein in follicular and diffuse large B-cell lymphomas. *Hematol Oncol Stem Cell Ther.* 2013;6(1):20–25.

DOI: 10.1093/labmed/lmaa027

Autophagy in Hematological Malignancies: Molecular Aspects in Leukemia and Lymphoma

Hassan Boustani, MSc,[1] Elahe Khodadi, MSc,[2] Minoo Shahidi, PhD[1]*

Laboratory Medicine 2021;52:16-23

DOI: 10.1093/labmed/lmaa027

ABSTRACT

The organization of the hematopoietic system is dependent on hematopoietic stem cells (HSCs) that are capable of self-renewal and multilineage differentiation to produce different blood cell lines. Autophagy has a central role in energy production and metabolism of the cells during starvation, cellular stress adaption, and removing mechanisms for aged or damaged organelles.

The role and importance of autophagy pathways are becoming increasingly recognized in the literature because these pathways can be useful in organizing intracellular circulation, molecular complexes, and organelles to meet the needs of various hematopoietic cells. There is supporting evidence in the literature that autophagy plays an emerging role in the regulation of normal cells and that it also has important features in malignant hematopoiesis. Understanding the molecular details of the autophagy pathway can provide novel methods for more effective treatment of patients with leukemia. Overall, our review will emphasize the role of autophagy and its different aspects in hematological malignant neoplasms.

Keywords: autophagy, hematological malignant neoplasms, hematopoiesis, molecular, leukemia, lymphoma

Abbreviations

ROS, reactive oxygen species; ATG, autophagy-related genes; HSPCs, high self-renewing precursor cells; LSCs, leukemic stem cells; MRD, minimal residual disease; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; miR, microRNA; FLT3, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; NPM, nucleophosmin 1; PML, promyelocytic leukemia; Akt, serine/threonine-protein kinase; LICs, leukemia-initiating cells; MLL-ENL, mixed lineage leukemia–eleven nineteen lysine-rich leukemia; ATG7, autophagy-related 7; ATG5, autophagy-related 5; AML1-ETO, acute myelogenous leukemia–1 eight-twenty-one oncoprotein; MLL, mixed-lineage leukemia; BM, bone marrow; HLX, H2.0-like homeobox transcription factor; AMPK, AMP-activated protein kinase; CML, chronic myeloid leukemia; BCR-ABL, breakpoint cluster region protein–Abelson murine leukemia; PIK-III, PtIns3P class III inhibitor; CCNG2, Cyclin-G2; MDS, myelodysplastic syndrome; MDS-RS, myelodysplastic syndrome with ring sideroblasts; ATG2B, autophagy-related protein 2 homolog B; GSKIP, GSK3B-interacting protein; ALL, acute lymphoid leukemia; B-ALL, acute lymphoid leukemia in B-cell precursors; T-ALL, acute lymphoid leukemia in thymocytes; BECN1, Beclin-1; pre-B ALL, pre-B acute lymphoblastic leukemia; CLL, chronic lymphoid leukemia; SLL, small lymphocytic lymphoma; B-CLL, B-cell chronic lymphocytic leukemia; TLR9, TolI-like receptor 9; IgM, immunoglobulin M; PI3K, phosphoinositide 3-kinase; PIK3R4, phosphoinositide-3-kinase regulatory subunit 4; DAPK1, death-associated protein kinase 1; CEBP-ATF6, CCAAT-enhancer-binding proteins–activating transcription factor 6; SLAMF1, signaling lymphocytic activation molecule family member 1; DLBCL, diffused large-cell B lymphoma; B-NHL, B–non-Hodgkin lymphoma; FL, follicular lymphoma; WHO, World Health Organization; BCL, B-cell lymphoma; CUL4B, Cullin-4B; JNK, c-Jun N-terminal kinases; MCL, mantle cell lymphoma; TG2, tissue transglutaminase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PLSCR1, phospholipid scramblase 1; hH, hedgehog; CXCR-4, C-X-C chemokine receptor type 4; BL, Burkitt lymphoma; MM, multiple myeloma; LC3, light chain 3; MDSCs, myeloid-derived suppressor cells; MALAT-1, metastasis-associated lung adenocarcinoma transcript–1; HMGB1, high mobility group box 1; mTORC, mammalian target of rapamycin complex; ALCL, anaplastic large-cell lymphoma; siRNA, small interfering RNA; HIF-1, hypoxia-inducible factor 1; HSCs, hematopoietic stem cells; FL, follicular lymphoma

[1]Department of Hematology and Blood Banking, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran, [2]Thalassemia & Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

*To whom correspondence should be addressed.
Shahidi.m@iums.ac.ir

Autophagy is a vesicular pathway in which the cellular components are moved to autophagosome vesicle and then will degenerate in lysosomes.[1] Autophagy is created in different conditions, such as nutrient- and energy-limiting conditions, endoplasmic reticulum stress, reactive oxygen species (ROS), hormonal imbalance, and exposure to microorganisms.[2] Autophagy plays an important role in cellular renewal of quiescent and differentiated cells, physiological processes, cell survival, and immune responses. This process is defective in inflammatory conditions, infections, cancer, neurodegenerative disorders, and aging.[3]

The results of studies[4] on yeast have revealed autophagy-related genes (ATG) that produce autophagosomes by a multistep process. The role of autophagy is complicated in cancer, depending on the stage, type, and driving oncogene in tumor cells. Study results[5,6] have shown the role of autophagy in tumor formation and metastasis in protection against apoptosis and support of cell survival. Also, the mechanisms involved in the therapy-resistance of tumor cells may occur due to autophagic response to therapy in tumor cells.

Hematopoiesis is a fully coordinated process in which hematopoietic stem pools and high self-renewing precursor cells (HSPCs) can form lymphoid and myeloid lineages. The HSPC pool decreases with aging, which results in a small number of HSPC clones that maintain hematopoiesis; with the decrease in blood-cell diversity, a phenomenon called clonal hematopoiesis occurs. Clonal proliferation in HSPCs that have a specific genetic mutation results in an increased risk of developing blood-based malignant neoplasms (**Figure 1**).[7]

Stem cell proliferation and differentiation in many tissues, including the hematopoietic system, is associated with dysregulation in malignant neoplasms. Leukemia cells have genetic and epigenetic defects that make them genetically distinct from normal blood cells. Hematopoietic cells are transformed at the stem cell stage and move towards the leukemic form. The initiation of leukemia, its progression, its resistance to treatment, and the recurrence of the disease are due to the proliferation of stem cells that are inherently resistant to conventional chemotherapy treatments.[8] Because leukemic stem cells (LSCs) also maintain minimal residual disease (MRD) and recur after treatment, a targeted approach is needed to eradicate them and provide sustainable treatment to patients.[9]

Early-onset autophagy increases the occurrence of hematological malignant neoplasms and increases the protumoral response that occurs in tumor growth stages by autophagy and in response to treatment modalities.[10] There is some evidence[11] that modulation of autophagy pathways can be a useful and effective therapeutic approach in patients with leukemia. Therefore, in this review, we discuss the autophagy pathway and its different molecular aspects in the development of hematological malignant neoplasms, including myeloid and lymphoid leukemia and lymphoma.

Autophagy in Myeloid Malignant Neoplasms

Acute Myeloid Leukemia (AML)

AML, the most common type of leukemia in older adults (80%), is characterized by arrest of myeloid differentiation and inappropriate proliferation and survival in leukemic blast cells associated with high clinical heterogeneity among individuals. Evidences[12,13] suggest that primary blasts in AML have lower autophagy gene levels, compared with nonleukemic cells or AML differentiation cells. Similarly, increased expression of autophagy receptor SQSMT1/p62 has been observed[14] during neutrophil differentiation in acute promyelocytic leukemia (APL) cells.

It has also been reported[14] that microRNA (miR)–17, -20, -93, and -106, which target SQSMT1/p62, are higher in mouse and human blast cells than in neutrophils. Increased expression of SQSMT1/p62 is thought[15,16] to prevent ubiquitinated protein accumulation. During the final differentiation of APL cells, it acts as a prosurvival cellular mechanism. Recent evidence[17] also suggests that SQSMT1/p62 is essential for cell growth and maintaining mitochondrial integrity. In fact, defects in SQSMT1/p62 impair the progression of myeloid leukemia and mitophagy in this type of malignant neoplasm.

Numerous articles in the literature support the idea that the role of autophagy in the progression of leukemia varies depending on the type of oncogene that can affect disease progression. The RET proto-oncogene is a tyrosine kinase that has recently been identified[18] as a key kinase in AML. Activated RET pathways lead to decreased autophagy and stability of leukemia-causing factors, such as mutant FMS-like tyrosine kinase 3 (FLT3). Also, RET inhibition leads to

Lab Medicine 2021;52;16–23 17
DOI: 10.1093/labmed/lmaa027

decreased FLT3 through autophagy. However, proteasome inhibitors increase FLT3 internal tandem duplication (FLT3/ITD) degeneration through autophagy.[19]

In contrast, inhibition of the FLT3-ITD mutation in AML cells, by impairing autophagy-dependent proliferation in vitro and in vivo, enhances a high level of basal autophagy. This FLT3-ITD–dependent autophagy depends on the extent of ATF4 transcription.[20] In AML cells mutated with nucleophosmin 1 (NPM), promyelocytic leukemia (PML) protein remains stable in the cytoplasm. PML insertion in the cytoplasm leads to serine/threonine-protein kinases (Akt) phosphorylation, which subsequently results in prosurvival autophagy.[21]

In studies of MLL-ENL mouse models, inhibition of autophagy by ATG7 deletion leads to decreased leukemia-initiating cells (LICs) and stronger survival in leukemic mice. These conditions led to an increase in ROS production, along with an increase in mitochondrial activity and cell death. Accordingly, blasts of this mouse model were reduced in peripheral blood due to increased apoptosis.[22]

Study results have shown that in the mixed lineage leukemia–eleven nineteen lysine-rich leukemia (MLL-ENL) mouse model, inhibition of autophagy by removing autophagy-related 7 (ATG7) or autophagy-related 5 (ATG5) results in more aggressive leukemia in vivo. In addition, these results showed that MLL-ENL cells with decreased autophagy activity lead to abnormal activity in mitochondria—specifically, proliferation and transformation.

In the acute myelogenous leukemia–1 eight-twenty-one oncoprotein (AML1-ETO) AML mouse model, autophagy inhibited LIC proliferation.[23] Mixed-lineage leukemia (MLL-Af9, or MA9) AML cells exhibit more autophagic activity than normal bone marrow (BM). However, degradation of Rb1cc1 or ATG5 does not affect the growth or survival of MA9-AML cells, in vitro and in vivo.[24] Study results[25] have shown that autophagy activity is required for malignancy but not required for maintenance of malignancy in MLL-Af9 AML cells.

The H2.0-like homeobox transcription factor (HLX), which shows increased expression in AML, is another factor involved in the regulation of hematopoietic differentiation. Increased expression of HLX has been reported to induce 5′ AMP-activated protein kinase (AMPK) activation and survival of AML cells through autophagy.[26] Overall, AML is a heterogeneous disease, and so autophagy acts as a tumor promoter and suppressor, depending on the AML subtype.

Chronic Myeloid Leukemia (CML)

CML accounts for 15% of all types of leukemia in adults, with displacement of t(22;9)(q34;q11) and expression of the breakpoint cluster region protein–Abelson murine leukemia viral oncogene homolog 1 (BCR-ABL) fusion protein, which has excessive tyrosine kinase activity. Study results[26] have shown that BCR-ABL1 induces MAPK15-dependent autophagy and causes cell transformation. However, other study results[27] have shown that BCR-ABL inhibits ATF5-dependent autophagy only in transformed cells. Lys05, the second generation of autophagy inhibitors, results in quiescence of CML stem cells and reduces the maturation of these cells. Also, Lys05 or PIK-III (PtIns3P class III inhibitor), when selectively combined with a tyrosine kinase inhibitor, reduced the number of primary CML LSCs. This finding suggests that this combination drug is associated with the destruction of stem cells in patients with CML.[27]

By using a certain type of cell culture and keeping the cells primarily at low oxygen concentration and subsequently exposed to oxygen, KML5 cells need autophagy only for commitment and not for proliferation.[28] Also, BIM1 expression as a transcription factor promotes CML progression to acute stages.[29] In fact, BIM1 inhibition increases Cyclin-G2 (CCNG2) expression and decreases tumor suppressor response in autophagy.

Myelodysplastic Syndrome (MDS)

MDS comprises a heterogeneous class of blood disorders.[30,31] It is divided into 4 main subgroups: MDS with single-lineage dysplasia, MDS with ring sideroblasts (MDS-RS), MDS with excess blasts, and MDS with isolated del(5q).

Numerous studies are underway to help researchers understand the effect of autophagy on the development and development of MDS. Increased expression of autophagy-related protein 2 homolog B (ATG2B) and GSK3B-interacting protein (GSKIP) caused changes in germline copy number in several myeloid malignant neoplasms.[32] However, study results[33] have shown that patients with MDS who harbor U2AF35 (S35F) mutation show an increase in pre-mRNA levels in ATG7 and have a distal cleft at the polyadenylation site, which reduce ATG7 expression and autophagy activity in cells. Nuclear red blood cells in patients with high-risk MDS have lower LC3B levels and greater mitochondrial deficiency; also, LC3B levels are

correlated with hemoglobin levels in these patients.[34] In addition, ATG3 expression is lower in patients with MDS than in healthy control individuals, and overexpression of ATG3 in the SKM-1 MDS cell line increases caspase-dependent autophagy activity and cell death.[35]

Autophagy in Lymphoid Malignancies

B- and T-lymphoblastic Leukemia/Lymphoma

Acute lymphoid leukemia (ALL) is the most common blood malignant neoplasm in children and accounts for 20% of adult leukemia cases.[36] This heterogeneous disorder involves different genomic changes, such as changes in the number and structure of chromosomes, and changes in the number of copies and mutations of DNA. This malignant neoplasm mainly occurs in B-cell precursors (B-ALL; 80%) and 20% in thymocytes (T-ALL). The results of a study[36] of 1 splice variant in Beclin-1 (BECN1) in the ALL 697 cell line with deletion in exon 11 have shown a decrease in autophagy induction in this malignant neoplasm.

We note that the ALL 697 cell line in the vicinity of Bafilomycin A1 decreased the graft in NOD/SCID mice. However, chromosomal translocation t (1;19) in pre-B acute lymphoblastic leukemia (pre-B ALL) in children induces autophagy in the face of rapamycin, leading to destruction of POLD1 DNA and RNA pol, and inhibits cell growth.[37] Also, using Torin-2 in pre-B ALL cells inhibits mTOR activity, enhances autophagy, and inhibits cell growth, which points to a tumor-suppressor role in autophagy.[38]

The role of autophagy in lymphoid malignant neoplasms is still controversial and may be observed in a specific subgroup. Further studies are needed to better understand the role of this recycling mechanism in leukemogenesis in the lymphoid lineage.

Chronic Lymphoid Leukemia (CLL)/Small Lymphocytic Lymphoma (SLL)

B-cell chronic lymphocytic leukemia (B-CLL)/SLL occurs predominantly in older adults and is characterized by inherent defects in cell death. This malignancy progresses slowly and shows clinical symptoms in afflicted patients. Study results[39] have shown that RNA inhibition of autophagy by targeting key autophagy genes in patients with CLL reduces cell survival.

Autophagy is also required for Toll-like receptor 9 (TLR9)–dependent secretion of immunoglobulin M (IgM) in a CLL-positive mouse model. High expression of phosphoinositide 3-kinase (PI3K), phosphoinositide-3-kinase regulatory subunit 4 (PIK3R4), and BECN1 is also associated with poor clinical outcome.[40]

Death-associated protein kinase 1 (DAPK1) is an autophagy-dependent gene 114; in rare cases of CLL, mutation in this gene increases HOXB7 binding to its promoter.[41] The loss of DAPK1 expression in CLL is due to a defect in the CCAAT-enhancer-binding proteins–activating transcription factor 6 (CEBP-ATF6) pathway function. Inhibition of DAPK1 reduces autophagy and increases the growth and proliferation of CLL cells.[42]

The dual role of autophagy in cancer has been proven in various studies. Signaling lymphocytic activation molecule family member 1 (SLAMF1) expression is associated with a favorable prognosis in patients with CLL and disappears in its invasive form.[43] Study results[43] have shown that SLAMF1 activates autophagy by stabilizing the BECN1-VPS34 complex; cells without SLAMF1 are less sensitive to autophagy-induced treatment.

Diffused Large Cell B Lymphoma (DLBCL)

DLBCL accounts for approximately 33% of B–non-Hodgkin lymphoma (B-NHL) and is the most common subgroup. DLBCL is an invasive lymphoma, de novo or the result of clinical progression of less-invasive B-NHL types (such as follicular lymphoma [FL] and CLL).

The 2016 World Health Organization (WHO) classification[44] introduced a new category of lymphomas, described as high-grade B-cell lymphoma with translocations related to MYC and B-cell lymphoma (BCL)–2 or BCL-6. BCL-2 directly inhibits autophagy by binding to BECN1; patients with decreased levels of BCL-2 show increased expression of BECN1, with favorable clinical outcomes.[45,46] Study results[47,48] have shown that in +/– BECN1 mice, the incidence of cancers such as lung, liver cancer, or B-cell lymphoma is higher. Other study results[49] have shown that inhibition of autophagic responses to DLBCL induced by BCL-6 may lead to lymphomagenesis. Also, a link between Cullin-4B (CUL4B) and autophagy and DLBCL progress

DOI: 10.1093/labmed/lmaa027

has been observed.[50] CUL4B regulates autophagy through c-Jun N-terminal kinases (JNK signaling); inhibition of cell proliferation by deletion mutation in CUL4B may inhibit autophagy-mediated cell survival. This finding highlights the complexity of autophagy pathways in the DLBCL.

Mantle Cell Lymphoma (MCL)

MCL is an invasive disease in older adults that accounts for approximately 5% of B-NHL. It is associated with high expression of tissue transglutaminase (TG2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).[51] Decreased expression of TG2 causes proliferation of cells in this disease. Also, TG2 regulates autophagy, and inhibition of autophagy by the ATG5 gene-silencing results in TG2 loss of expression. In addition, ATG5-free cells reproduce less slowly than cells with autophagy.

Phospholipid scramblase 1 (PLSCR1) is a proapoptotic gene that, through 9-cis-retinoic and interferon-α, decreases its expression in MCL cells.[52,53] Also, PLSCR1 inhibits autophagy and reduces the survival of malignant cells in MCL.[54] In contrast, the hedgehog (hH) pathway in MCL cells increases the penetration of malignant cells into the BM. Inhibition of the hH pathway using LDE225 increased C-X-C chemokine receptor type 4 (CXCR-4) expression and, consequently, ROS increased autophagy activity and cell survival.[55]

Burkitt Lymphoma (BL)

BL is an invasive disease in immature B cells that is known by the rearrangement of the *MYC* gene, with early B cell markers testing positive for the disease and the involved cells having a high mitosis rate. BL is a rare disease that accounts for 1% to 2% of B-NHL cases in adults and 30% of pediatric lymphoma cases. The findings of a study on e-myc transgenic mice[56] have shown that dysfunction of lysosomes using chloroquine has prevented lymphomagenesis, suggesting the association of lymphomagenesis with autophagy in these patients.

Multiple Myeloma (MM)

MM is a plasma B-cell cancer that invades the BM, is associated with high genomic and phenotypic diversity, and is observed in elderly patients.[57] Intron 6 of ATG5 on chromosome 6q21 is a risk loci associated with a high probability of developing MM.[58,59] High expression of BECN1 or microtubule-associated protein 1A/1B-light chain 3 (LC3) is also associated with favorable clinical outcomes.[60] This finding suggests that autophagic dysfunction is crucial to the progression of MM disease.

However, findings from other studies indicate that activation of autophagy is essential for MM survival. A recent publication[61] reports that the role of myeloid-derived suppressor cells (MDSCs) in enhancing survival and proliferation of MM cells by activating the AMPK pathway has been demonstrated and that the prosurvival effect on AMPK may be due to autophagy induction. It has also been shown[62] in BM cells of patients with MM that overexpression of long noncoding RNA metastasis-associated lung adenocarcinoma transcript–1 (MALAT-1) and high mobility group box 1 (HMGB1) results in autophagy and survival.

CHE-1 protein, which is antiapoptotic, interacts with RNA polymerase II and regulates its expression. Expression of this protein is associated with MM progression and is essential for cell growth and survival. CHE-1 is phosphorylated by cellular stress and, by binding to the Redd1 and Deptor promoters, increases their expression and thereby decreases mammalian target of rapamycin complex (mTORC) activity. Expression of CHE-1 induces autophagy activity by induction of mTORC1 and mTORC2, indicating its association with autophagy in MM.[63] The results of several studies, such as Hoang et al,[64] have shown that inhibition of autophagy decreases cell survival in MM. However, other study results[65] point to the role of cell death and its association with autophagy in MM cells.

Anaplastic Large Cell Lymphoma (ALCL)

ALCL accounts for 1% to 3% of adult T-NHL lymphoma and 15% of pediatric lymphoma.[66] The role of autophagy in the development of ALCL has not been determined, to our knowledge. The results of a study[67] using derived cell lines have shown that inhibition of autophagy by small interfering RNA (siRNA) binding to ATG7 or chloroquine has no effect on cell viability.

FL

FL accounts for approximately 20% of B-NHL and is the second most common form of lymphoma worldwide.[68,69] Due to the role of autophagy in the regulation of B-cells, study results[70] have shown that patients with FL had a significant decrease in SQSMT1/p62 and LC3 levels, compared with reactive B-cells; this finding indicates increased autophagy activity in FL. The impairment of autophagy regulation in FL is not associated with overexpression

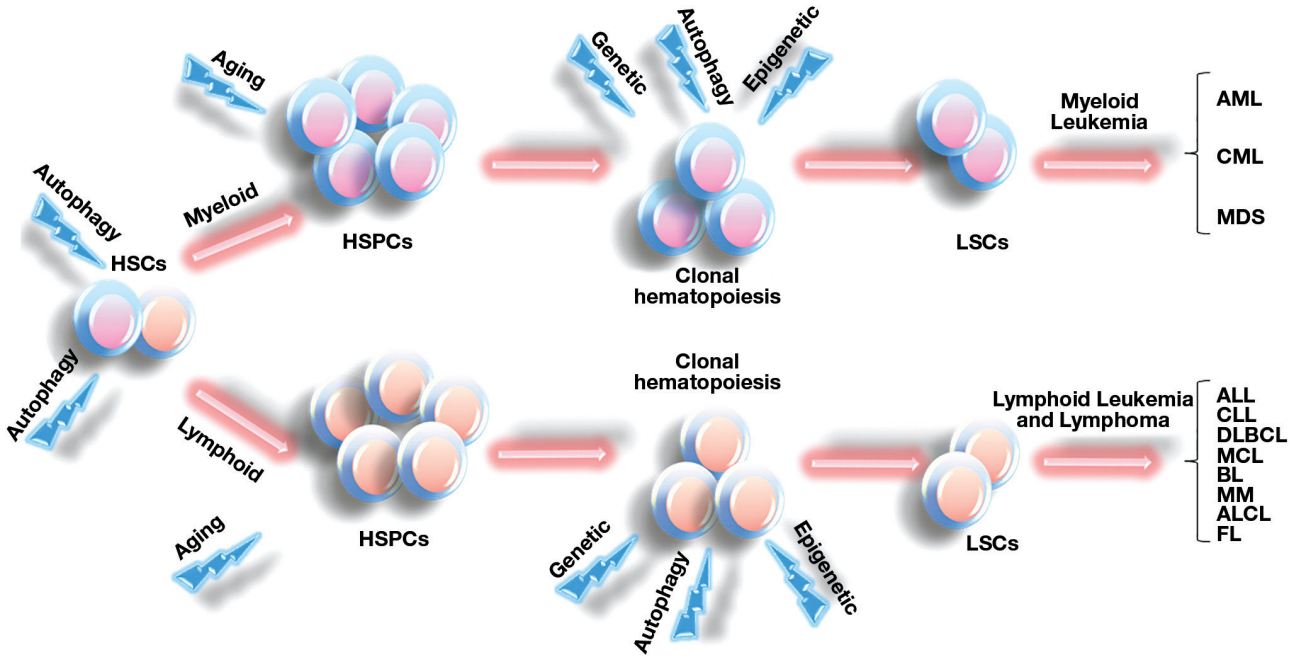


Figure 1

The role of autophagy in normal and malignant cells. Normal high self-renewing precursor cells (HSPCs) can form lymphoid and myeloid lineages by autophagy. Aging results in a decrease and in clonal hematopoiesis of HSPCs. Genetic, epigenetic, and autophagy defects make leukemic stem cells (LSCs) in lymphoid and myeloid cells, which result in myeloid and lymphoid leukemias and lymphomas. HSCs indicates hematopoietic stem cells; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoid leukemia; CLL, chronic lymphoid leukemia; DLBCL, diffused large-cell B lymphoma; MCL, mantle cell lymphoma; BL, Burkitt lymphoma; MM, multiple myeloma; ALCL, anaplastic large-cell lymphoma; FL, follicular lymphoma.

of BCL-2. FL is associated with overexpression of LC3A and overexpression of hypoxia-inducible factor 1 (HIF-1), indicating its association with hypoxia, and has autophagic activity in FL.[71]

Conclusions

Although the role of autophagy in stem-cell transformation and hematological malignant neoplasms has been investigated in many studies, its role in treatment strategies for these disorders is controversial. Also, autophagy modulation has been reported[59] to play a role in outcomes for hematopoietic malignant neoplasms. Understanding the exact molecular mechanism of autophagy in normal and malignant hematopoiesis can help to provide better treatment strategies for patients. Further, each autophagy pathway in every lineage has a specific characteristic, so targeting theses pathways can result in specific strategies for each type of malignant neoplasms. However, further studies are needed to answer remaining questions in this field. LM

References

1. Klionsky DJ, Codogno P. The mechanism and physiological function of macroautophagy. *J Innate Immun.* 2013;5(5):427–433.
2. Kroemer G, Mariño G, Levine B. Autophagy and the integrated stress response. *Mol Cell.* 2010;40(2):280–293.
3. Choi AMK, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med.* 2013;368(7):651–662.
4. Mizushima N, Yoshimori T, Ohsumi Y,. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol*. 2010;27:107–132.
5. Strohecker AM, White E. Autophagy promotes BrafV600E-driven lung tumorigenesis by preserving mitochondrial metabolism. *Autophagy.* 2014;10(2):384–385.
6. Guo JY, White E. Autophagy is required for mitochondrial function, lipid metabolism, growth, and fate of KRAS(G12D)-driven lung tumors. *Autophagy.* 2013;9(10):1636–1638.

DOI: 10.1093/labmed/lmaa027

7. Haybar H, Shahrabi S, Ghanavat M, Khodadi E. Clonal hematopoiesis: genes and underlying mechanisms in cardiovascular disease development. *J Cell Physiol.* 2019;234(6):8396–8401.
8. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med.* 1997;3(7):730–737.
9. Koschade SE, Brandts CH. Selective autophagy in normal and malignant hematopoiesis. *J Mol Biol.* 2020;432(1):261–282.
10. Salio M, Puleston DJ, Mathan TSM, et al. Essential role for autophagy during invariant NKT cell development. *Proc Natl Acad Sci U S A.* 2014;111(52):E5678–E5687.
11. Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat Rev Cancer.* 2008;8(1):11–23.
12. Jin J, Britschgi A, Schläfli AM, et al. Low autophagy (ATG) gene expression is associated with an immature AML blast cell phenotype and can be restored during AML differentiation therapy. *Oxid Med Cell Longev.* 2018;2018:1482795.
13. Watson AS, Riffelmacher T, Stranks A, et al. Autophagy limits proliferation and glycolytic metabolism in acute myeloid leukemia. *Cell Death Dis*. 2015;1:15008.
14. Meenhuis A, van Veelen PA, de Looper H, et al. MiR-17/20/93/106 promote hematopoietic cell expansion by targeting sequestosome 1-regulated pathways in mice. *Blood.* 2011;118(4):916–925.
15. Trocoli A, Bensadoun P, Richard E, et al. p62/SQSTM1 upregulation constitutes a survival mechanism that occurs during granulocytic differentiation of acute myeloid leukemia cells. *Cell Death Differ.* 2014;21(12):1852–1861.
16. Ségal-Bendirdjian E, Tschan MP, Reiffers J, Djavaheri-Mergny M. Pro-survival role of p62 during granulocytic differentiation of acute myeloid leukemia cells. *Mol Cell Oncol.* 2014;1(4):e970066.
17. Nguyen TD, Shaid S, Vakhrusheva O, et al. Loss of the selective autophagy receptor p62 impairs murine myeloid leukemia progression and mitophagy. *Blood.* 2019;133(2):168–179.
18. Rudat S, Pfaus A, Cheng YY, et al. RET-mediated autophagy suppression as targetable co-dependence in acute myeloid leukemia. *Leukemia.* 2018;32(10):2189–2202.
19. Larrue C, Saland E, Boutzen H, et al. Proteasome inhibitors induce FLT3-ITD degradation through autophagy in AML cells. *Blood.* 2016;127(7):882–892.
20. Heydt Q, Larrue C, Saland E, et al. Oncogenic FLT3-ITD supports autophagy via ATF4 in acute myeloid leukemia. *Oncogene.* 2018;37(6):787–797.
21. Zou Q, Tan S, Yang Z, et al. NPM1 mutant mediated PML delocalization and stabilization enhances autophagy and cell survival in leukemic cells. *Theranostics.* 2017;7(8):2289–2304.
22. Sumitomo Y, Koya J, Nakazaki K, et al. Cytoprotective autophagy maintains leukemia-initiating cells in murine myeloid leukemia. *Blood.* 2016;128(12):1614–1624.
23. Man N, Tan Y, Sun X-J, et al. Caspase-3 controls AML1-ETO–driven leukemogenesis via autophagy modulation in a ULK1-dependent manner. *Blood.* 2017;129(20):2782–2792.
24. Chen X, Clark J, Wunderlich M, et al. Autophagy is dispensable for *Kmt2a/Mll-Mllt3/Af9* AML maintenance and anti-leukemic effect of chloroquine. *Autophagy.* 2017;13(5):955–966.
25. Liu Q, Chen L, Atkinson JM, Claxton DF, Wang H-G. Atg5-dependent autophagy contributes to the development of acute myeloid leukemia in an MLL-AF9-driven mouse model. *Cell Death Dis.* 2016;7(9):e2361.
26. Piragyte I, Clapes T, Polyzou A, et al. A metabolic interplay coordinated by HLX regulates myeloid differentiation and AML through partly overlapping pathways. *Nat Commun.* 2018;9(1):3090.
27. Baquero P, Dawson A, Mukhopadhyay A, et al. Targeting quiescent leukemic stem cells using second generation autophagy inhibitors. *Leukemia.* 2019;33(4):981–994.
28. Ianniciello A, Dumas P-Y, Drullion C, et al. Chronic myeloid leukemia progenitor cells require autophagy when leaving hypoxia-induced quiescence. *Oncotarget.* 2017;8(57):96984–96992.
29. Mourgues L, Imbert V, Nebout M, et al. The BMI1 polycomb protein represses cyclin G2-induced autophagy to support proliferation in chronic myeloid leukemia cells. *Leukemia.* 2015;29(10):1993–2002.
30. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol.* 1982;51(2):189–199.
31. Cogle CR, Saki N, Khodadi E, Li J, Shahjahani M, Azizidoost S. Bone marrow niche in the myelodysplastic syndromes. *Leuk Res.* 2015;39(10):1020–1027.
32. Saliba J, Saint-Martin C, Di Stefano A, et al. Germline duplication of ATG2B and GSKIP predisposes to familial myeloid malignancies. *Nat Genet.* 2015;47(10):1131–1140.
33. Park SM, Ou J, Chamberlain L, et al. U2AF35(S34F) promotes transformation by directing aberrant *ATG7* pre-mRNA 3′ end formation. *Mol Cell.* 2016;62(4):479–490.
34. Jiang H, Yang L, Guo L, Cui N, Zhang G, Liu C, et al. Impaired mitophagy of nucleated erythroid cells leads to anemia in patients with myelodysplastic syndromes. *Oxid Med Cell Longev* 2018;2018;6328051.
35. Zhuang L, Ma Y, Wang Q, et al. Atg3 Overexpression enhances bortezomib-induced cell death in SKM-1 cell. *PLoS One.* 2016;11(7):e0158761.
36. Niu Y-N, Liu Q-Q, Zhang S-P, et al. Alternative messenger RNA splicing of autophagic gene Beclin 1 in human B-cell acute lymphoblastic leukemia cells. *Asian Pac J Cancer Prev.* 2014;15(5):2153–2158.
37. Wang Z, Xu F, Yuan N, et al. Rapamycin inhibits pre-B acute lymphoblastic leukemia cells by downregulating DNA and RNA polymerases. *Leuk Res.* 2014;38(8):940–947.
38. Simioni C, Cani A, Martelli AM, et al. Activity of the novel mTOR inhibitor Torin-2 in B-precursor acute lymphoblastic leukemia and its therapeutic potential to prevent Akt reactivation. *Oncotarget.* 2014;5(20):10034–10047.
39. El-Khoury V, Pierson S, Szwarcbart E, et al. Disruption of autophagy by the histone deacetylase inhibitor MGCD0103 and its therapeutic implication in B-cell chronic lymphocytic leukemia. *Leukemia.* 2014;28(8):1636–1646.
40. Kristensen L, Kristensen T, Abildgaard N, et al. High expression of PI3K core complex genes is associated with poor prognosis in chronic lymphocytic leukemia. *Leuk Res.* 2015;39(6):555–560.
41. Raval A, Tanner SM, Byrd JC, et al. Downregulation of *death-associated protein kinase 1 (DAPK1)* in chronic lymphocytic leukemia. *Cell.* 2007;129(5):879–890.
42. Gade P, Kimball AS, DiNardo AC, et al. Death-associated protein kinase-1 expression and autophagy in chronic lymphocytic leukemia are dependent on activating transcription factor-6 and CCAAT/enhancer-binding protein-β. *J Biol Chem.* 2016;291(42):22030–22042.
43. Bologna C, Buonincontri R, Serra S, et al. SLAMF1 regulation of chemotaxis and autophagy determines CLL patient response. *J Clin Invest.* 2016;126(1):181–194.
44. Friedberg JW. How I treat double-hit lymphoma. *Blood.* 2017;130(5):590–596.
45. Nicotra G, Mercalli F, Peracchio C, et al. Autophagy-active beclin-1 correlates with favourable clinical outcome in non-Hodgkin lymphomas. *Mod Pathol.* 2010;23(7):937–950.
46. Huang J-J, Zhu Y-J, Lin T-Y, Jiang W-Q, Huang H-Q, Li Z-M. Beclin 1 expression predicts favorable clinical outcome in patients with diffuse large B-cell lymphoma treated with R-CHOP. *Hum Pathol.* 2011;42(10):1459–1466.
47. Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci U S A.* 2003;100(25):15077–15082.

48. Qu X, Yu J, Bhagat G, et al. Promotion of tumorigenesis by heterozygous disruption of the *beclin 1* autophagy gene. *J Clin Invest.* 2003;112(12):1809–1820.
49. Bertolo C, Roa S, Sagardoy A, et al. *LITAF*, a BCL6 target gene, regulates autophagy in mature B-cell lymphomas. *Br J Haematol.* 2013;162(5):621–630.
50. Li Y, Zhou X, Zhang Y, et al. CUL4B regulates autophagy via JNK signaling in diffuse large B-cell lymphoma. *Cell Cycle.* 2019;18(4):379–394.
51. Zhang H, Chen Z, Miranda RN, Medeiros LJ, McCarty N. TG2 and NF-κB signaling coordinates the survival of mantle cell lymphoma cells via IL6-mediated autophagy. *Cancer Res.* 2016;76(21):6410–6423.
52. Zhou Q, Zhao J, Al-Zoghaibi F, et al. Transcriptional control of the human plasma membrane phospholipid scramblase 1 gene is mediated by interferon-alpha. *Blood.* 2000;95(8):2593–2599.
53. Zhao K-W, Li X, Zhao Q, et al. Protein kinase Cδ mediates retinoic acid and phorbol myristate acetate-induced phospholipid scramblase 1 gene expression: its role in leukemic cell differentiation. *Blood.* 2004;104(12):3731–3738.
54. Mastorci K, Montico B, Faè DA, et al. Phospholipid scramblase 1 as a critical node at the crossroad between autophagy and apoptosis in mantle cell lymphoma. *Oncotarget.* 2016;7(27):41913–41928.
55. Zhang H, Chen Z, Neelapu SS, Romaguera J, McCarty N. Hedgehog inhibitors selectively target cell migration and adhesion of mantle cell lymphoma in bone marrow microenvironment. *Oncotarget.* 2016;7(12):14350–14365.
56. Maclean KH, Dorsey FC, Cleveland JL, Kastan MB. Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis. *J Clin Invest.* 2008;118(1):79–88.
57. Kuehl WM, Bergsagel PL. Molecular pathogenesis of multiple myeloma and its premalignant precursor. *J Clin Invest.* 2012;122(10):3456–3463.
58. Mitchell JS, Li N, Weinhold N, et al. Genome-wide association study identifies multiple susceptibility loci for multiple myeloma. *Nat Commun.* 2016;7:12050.
59. Went M, Sud A, Försti A, et al; PRACTICAL consortium. Identification of multiple risk loci and regulatory mechanisms influencing susceptibility to multiple myeloma. *Nat Commun.* 2018;9(1):3707.
60. Jung G, Roh J, Lee H, et al. Autophagic markers BECLIN 1 and LC3 are associated with prognosis of multiple myeloma. *Acta Haematol.* 2015;134(1):17–24.
61. De Veirman K, Menu E, Maes K, et al. Myeloid-derived suppressor cells induce multiple myeloma cell survival by activating the AMPK pathway. *Cancer Lett.* 2019;442:233–241.
62. Gao D, Lv A-E, Li H-P, Han D-H, Zhang Y-P. LncRNA MALAT-1 elevates HMGB1 to promote autophagy resulting in inhibition of tumor cell apoptosis in multiple myeloma. *J Cell Biochem.* 2017;118(10):3341–3348.
63. Desantis A, Bruno T, Catena V, et al. Che-1-induced inhibition of mTOR pathway enables stress-induced autophagy. *EMBO J.* 2015;34(9):1214–1230.
64. Hoang B, Benavides A, Shi Y, Frost P, Lichtenstein A. Effect of autophagy on multiple myeloma cell viability. *Mol Cancer Ther.* 2009;8(7):1974–1984.
65. Lamy L, Ngo VN, Emre NCT, et al. Control of autophagic cell death by caspase-10 in multiple myeloma. *Cancer Cell.* 2013;23(4):435–449.
66. Turner SD, Lamant L, Kenner L, Brugières L. Anaplastic large cell lymphoma in paediatric and young adult patients. *Br J Haematol.* 2016;173(4):560–572.
67. Mitou G, Frentzel J, Desquesnes A, et al. Targeting autophagy enhances the anti-tumoral action of crizotinib in ALK-positive anaplastic large cell lymphoma. *Oncotarget.* 2015;6(30):30149–30164.
68. Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. *J Clin Invest.* 2012;122(10):3424–3431.
69. Gravelle P, Do C, Franchet C, et al. Impaired functional responses in follicular lymphoma $CD8^{+}TIM\text{-}3^{+}$ T lymphocytes following TCR engagement. *Oncoimmunology.* 2016;5(10):e1224044.
70. McCarthy A, Marzec J, Clear A, et al. Dysregulation of autophagy in human follicular lymphoma is independent of overexpression of BCL-2. *Oncotarget.* 2014;5(22):11653–11668.
71. Giatromanolaki A, Koukourakis MI, Pouliliou S, et al. Overexpression of LC3A autophagy protein in follicular and diffuse large B-cell lymphomas. *Hematol Oncol Stem Cell Ther.* 2013;6(1):20–25.

DOI: 10.1093/labmed/lmaa027

Mesenchymal Stem Cells in COVID-19: A Journey from Bench to Bedside

Kamal Kant Sahu, MD,[1*] *Ahmad Daniyal Siddiqui, MD,*[1] *Jan Cerny, MD, PhD*[2]

Laboratory Medicine 2021;52:24-35

DOI: 10.1093/labmed/lmaa049

ABSTRACT

The COVID-19 pandemic has led to a major setback in both the health and economic sectors across the globe. The scale of the problem is enormous because we still do not have any specific anti-SARS-CoV-2 antiviral agent or vaccine. The human immune system has never been exposed to this novel virus, so the viral interactions with the human immune system are completely naive. New approaches are being studied at various levels, including animal in vitro models and human-based studies, to contain the COVID-19 pandemic as soon as possible. Many drugs are being tested for repurposing, but so far only remdesivir has shown some positive benefits based on preliminary reports, but these results also need further confirmation via ongoing trials. Otherwise, no other agents have shown an impactful response against COVID-19.

Recently, research exploring the therapeutic application of mesenchymal stem cells (MSCs) in critically ill patients suffering from COVID-19 has gained momentum. The patients belonging to this subset are most likely beyond the point where they could benefit from an antiviral therapy because most of their illness at this stage of disease is driven by inflammatory (over)response of the immune system. In this review, we discuss the potential of MSCs as a therapeutic option for patients with COVID-19, based on the encouraging results from the preliminary data showing improved outcomes in the progression of COVID-19 disease.

Keywords: Stem cells, COVID-19, SARS-CoV-2 Virus, pandemic, vaccine, clinical trials

As of June 3, 2020, there were 6,551,389 global COVID-19 cases with 386,196 deaths. Although approximately 98% of patients experience mild disease, 2% experience severe disease often requiring critical care support.[1,2]

Abbreviations:

MSCs, mesenchymal stem cells; FDA, U.S. Food and Drug Administration; NK cells, natural killer cells; IL, interleukin; TNF, tumor necrosis factor; ARDS, acute respiratory distress syndrome; ICU, intensive care unit; ACE-2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease, serine 2; DC, dendritic cell; BM, bone marrow; AT, adipose tissue; UC, umbilical cord; IV, intravenous; UC-MSCs, umbilical cord mesenchymal stem cells; MHC, major histocompatibility complex; SCE, stem cell educator; AE, adverse event; SAE, serious adverse event; CT, computed tomography; PSI, patient safety indicators; MSCs, mesenchymal stem cells; PaO2, partial pressure of oxygen; FiO2, fraction of inspired oxygen; PCT, procalcitonin; SAA, serum amyloid A; CRP, C-reactive protein; BM-MSC, bone marrow mesenchymal stem cells; RT-PCR, real-time polymerase chain reaction

[1]Department of Hematology and Oncology, Department of Internal Medicine, Saint Vincent Hospital, 123 Summer Street, Worcester, Massachusetts, [2]Division of Hematology and Oncology, Department of Medicine, UMass Memorial Health Care, University of Massachusetts Medical School, Worcester, Massachusetts

*To whom correspondence should be addressed.
drkksahu85@gmail.com

To date, physicians and intensivists treating patients with COVID-19 have limited treatment options in the absence of an effective antiviral agent or vaccine.[3-5] Therapeutic options in the form of convalescent plasma therapy, remdesivir, tocilizumab, and repurposing of various drugs are being explored.[6-10] Recently, a few studies have examined the role of mesenchymal stem cells (MSCs) in critically ill patients with COVID-19. These studies have shown some early promise as therapeutic possibilities for the treatment of severe COVID-19.[11-14]

Basic Principles of MSC Therapy

In multiple and mostly regenerative clinical settings, MSCs have been evaluated to either treat or prevent a disease or condition characterized by tissue damage. Regenerative treatment models have so far suggested the following major methods of repair:[15,16] anti-inflammatory effect; stem cell homing to damaged networks, with recruitment of other cells; inhibition of apoptosis; and differentiation capability. The molecules and exosomes emitted from stem cells promote tissue healing and regeneration. In addition to the

direct effect, stem cells also possess a paracrine function that works by releasing the soluble factors termed *stem cell secretomes.*[17,18] This property allows the systemic distribution of the positive immunomodulatory and regenerative effects of MSCs throughout the body, thereby ensuring a systemic effect in addition to local modulation.[18]

Stem Cell Therapies and Concerns

The role of MSCs in regenerative medicine has been explored in the past. Many studies have shown the beneficial effects of MSC-based therapies.[14,16,19] Such therapies have been studied in various neurological disorders, endocrinopathies, and bone and cartilage diseases. Contrary to these results, some studies have showed that MSCs may promote cancer pathogenesis.[20,21] Therefore, the role of MSCs in regenerative medicine and their therapeutic potential needs more study and clarification.[22] There is thus a constant effort from research bodies and other agencies such as the U.S. Food and Drug Administration (FDA), the International Society for Cellular Therapy, and the International Society for Stem Cell Research to actively monitor stem cell clinic industries across the globe to minimize unapproved and unproven cell-based therapies.[23,24] With regard to the current pandemic, the main challenge is the novelty of the disease, its unfamiliar pathophysiology, and the lack of an anti-SARS-CoV-2 antiviral agent or vaccine. Realizing the pandemic as a time-sensitive matter, the FDA has been relatively liberal in clearing pathways to study MSCs and natural killer (NK) cells for their potential immune activity in response to COVID-19.[25,26]

Rationale of Using MSCs in COVID-19

Research has shown that MSCs have specific cytokines that drive immunomodulation, which may be useful against SARS-CoV-2.[27] The available literature so far suggests that the hallmark of SARS-CoV-2 infection is a cytokine-induced storm. SARS-CoV-2 induces an acute release of cytokines such as granulocyte colony-stimulating factor, IP10, Monocyte Chemoattractant Protein-1, interleukin (IL)-2/6/7, and tumor necrosis factor (TNF) in large amounts.[28,29] These cytokines lead to increased vasculature permeability, pulmonary edema, vascular congestion, and impairment of air exchange across the membranes, and in severe cases they may lead to acute respiratory distress syndrome (ARDS) and death.

Recently, Huang et al[30] studied the cytokine profile of 41 patients with COVID-19. They found higher plasma levels of proinflammatory cytokines in patients in the intensive care unit (ICU) than in patients who were not. Chen, Wu, et al[31] found markedly lower absolute numbers of T lymphocytes, CD4+ T cells, and CD8+ T cells in patients with severe cases.[31] The patients with high cytokine levels, lower CD4+ T cells, and lower CD8+ T cells became more sick, required ICU care, and had a higher likelihood of developing ARDS.

Current efforts with MSC therapeutics are focusing on the capability of MSCs to abort or minimize this cytokine storm, thereby reducing lung damage and promoting the restoration of tissue function through their inherent reparative properties.[32] The MSCs express angiotensin-converting enzyme 2 (ACE-2) and transmembrane protease serine 2 (TMPRSS2) cells and have the potential to induce mature dendritic cells (DCs) to novel Jagged-2 dependent regulatory DCs that possess an immunosuppressive capacity to generate specific immune tolerance and diminish Th2-type inflammation.[33,34] The MSCs also help in the preferable differentiation of human CD34+ cells to regulatory DCs over classical DCs.[35] Based on these immunomodulatory functions, Leng et al[11] found MSC infusion beneficial in their patients with COVID-19. Similarly, Zhao[36] also suggested that MSCs could help patients who are critically ill with COVID-19.

Biological Characteristics of MSCs and Identification of the Best Stem Cell Source

Stem cells derived from various tissues in the body are being evaluated for therapies in numerous degenerative disorders.[16,37] The common stem cells used in clinical practice originate from bone marrow (BM), adipose tissue (AT), amnion, the umbilical cord (UC), dental pulp, menstrual blood, the buccal fat pad, and fetal liver.[37] The MSCs hold much promise to change the dynamics of incurable diseases for

DOI: 10.1093/labmed/lmaa049

many reasons: (i) easy accessibility, (ii) multipotency, (iii) ease of expansion to required clinical volume, (iv) storage potential for repetitive therapeutic usage, (v) immune evasive property indicating a minimal chance of rejection with allogeneic MSCs, and (vi) easy route of administration (via intravenous [IV]).

For patients with COVID-19, among the various stem cells, UC mesenchymal stem cells (UC-MSCs) have recently gained more attention because of their ready availability, compatibility, potency, and plasticity:[19,38-41]

i. When compared with BM, UC harvests have a higher concentration of stem cells.
ii. The UC-MSCs have a faster doubling time.
iii. A higher proliferation rate of UC-MSCs allows for a scalable expansion that may benefit a larger population of critically ill patients.
iv. The harvesting tissue for UC-MSCs is a byproduct during delivery and does not require any invasive procedure unlike BM stem cell extraction.
v. The UC-MSCs have the advantage of being immune tolerant because of low expression of major histocompatibility complex (MHC) class I and no expression of MHC class II. These characteristics allow the use of even allogeneic MSCs.

Similarly, another potential source of MSCs with an advantage over other harvesting sites is AT. Using AT-derived MSCs has the following benefits:[38,39]

i. Easily accessible site for extraction that poses minimum discomfort to the donor.
ii. Comparatively easy to isolate MSCs from the harvested AT.
iii. Comparatively, a higher fraction of MSCs can be extracted from harvested AT compared with BM.
iv. Comparatively, a higher success rate of isolating MSCs from AT than UC.

Reviving COVID-19-Induced Lung Damage via MSCs

Studies on animal models have shown that pneumocyte type II cells support coronavirus replication better than pneumocyte type I cells and alveolar macrophages.[42,43] In addition, postmortem histopathology examination of lung tissue from patients with COVID-19 has shown significant lung damage with evidence of diffuse alveolar damage, type II pneumocyte hyperplasia, and intra-alveolar fibrinous exudates.[44] Recent studies have provided ample evidence that stem cells promote lung tissue healing and regeneration by differentiating to pulmonary epithelial cells. Once injected intravenously, a significant amount of MSCs accumulate in the lung and exhibit an immunomodulatory effect, thereby protecting the alveolar epithelial cells, restoring the pulmonary alveolar niche, preventing fibrosis, and improving overall pulmonary function.[45,46] This phenomenon may benefit critically ill patients with COVID-19 and help them recover from ARDS, pulmonary edema, and diffuse alveolar damage.

Clinical Use of MSCs in COVID-19: Paucity of Data

The COVID-19 pandemic is evolving, and we are still in the learning phase. There is a lack of impactful data on MSC therapeutics for COVID-19. Physicians can extrapolate the potential clinical benefits of MSCs in COVID-19 pneumonia based on studies from previous viral outbreaks with positive outcomes.[47] Chen et al infused MSCs extracted from allogeneic menstrual blood from healthy female donors into 17 patients with H7N9-induced ARDS. They found a significant mortality benefit in the MSC-infused arm (17.6% died) as compared with the control arm (54.5% died). The major limitations of the study were the small sample size, high attrition rate, and not-ideal comparison sample because patients were receiving other drugs as well.

Because H7N9 and SARS-CoV-2 share similar complications—ARDS, hypoxic respiratory failure, severe inflammation, overt immune response, and multiorgan dysfunction syndrome—MSCs therapy may be beneficial for patients with COVID-19 pneumonia as well.[27,28] A recent meta-analysis on MSC use in ARDS (study period 1990 to March 31, 2020)[7] showed an improvement in radiographic shadows, pulmonary function, and biochemical marker levels. This meta-analysis also showed a mortality benefit with the use of MSCs but not to a significant level.

With regard to COVID-19, Leng et al[11] recently used BM-derived MSCs intravenously in 7 patients with COVID-19 (1 critically severe illness, 4 severe illnesses, and 2

common illnesses). Significant clinical benefit was noted in all patients with symptomatic improvement and reduced oxygen requirement after 2 to 4 days of MSC transplantation. Mass cytometry and cytokine analysis of the patients' peripheral blood also showed disappearance of overactivated T cells and NK cells, an increase in anti-inflammatory cytokines like IL-10, and a decrease in proinflammatory cytokines like TNF-alpha. However, the small sample size, the lack of a control arm, patients who were also receiving other drugs, and a patient cohort with only 1 patient with critical serious illness are possible limitations to consider before analyzing the results.

The above-discussed studies regarding the use of MSCs in COVID-19 have provided a much-needed clinical platform for further research on MSCs in COVID-19 and other viral disorders. Therapy using MSC in high-risk populations including pregnant women, patients with human immunodeficiency virus, patients with malignancies, and transplant recipients would need further refinement before being executed.[48-52] In addition, it is too early to suggest that MSCs are safe to use in patients with COVID-19 and requires further confirmation.[53,54]

Current Literature on Ongoing Trials of MSCs for COVID-19

Recently, clinical trials studying various aspects of MSC use in COVID-19 are underway in several clinical phases (**Figure 1**). China and the United States are the 2 leading countries studying cell-based therapy–related clinical trials, from which some reports have been published as well (**Table 1**).[55,56] As of April 21, 2020, 40 clinical trials were active across the globe (http://www.chictr.org.cn/index.aspx and https://clinicaltrials.gov/). Zhongnan Hospital in China and its collaborator, Tuohua Biological Technology Co. Ltd., are studying UC-MSC treatment (IV on day 1, day 3, day 5, and day 7) in patients with serious and critical pneumonia. The primary outcome is to study the improvement in oxygenation index on day 14 after enrollment (NCT04269525). Similarly, Renmin Hospital of Wuhan University is planning to evaluate the safety and efficacy of allogeneic human dental pulp MSCs in severe pneumonia caused by COVID-19 (ChiCTR2000031319). Elsewhere, scientists from other countries including the United Kingdom, Brazil, Jordan, and France are conducting similar MSC therapy clinical trials for COVID-19.

Side Effects and Concerns Over Practical Usability During COVID-19 Pandemic

To prove MSC therapy successful, in addition to evaluating its efficacy it is equally important to assess other practical aspects of community use and accessibility.[57,58] With regard to MSCs in COVID-19, although the experience so far has been encouraging, the key barriers still include the following:[59-61]

i. Data to date are preliminary and based on compassionate use of MSCs, with final results from clinical trials still pending.
ii. The downstream effects of MSCs on the lungs are unclear and unknown. For example, we lack an understanding of MSC impact on the expression of ACE-2 and TMPRSS2, which are known facilitators of viral entry into cells and subsequent replication (**Figure 2**).
iii. Similarly, there is a differential expression of ACE-2 receptors in various organ systems. It would be noteworthy to determine whether there would be a difference in the recovery/response of different organs upon MSC infusion.
iv. There is concern over the commercialization and subsequent abuse of unproven cell-based therapies by unauthorized stem cell clinics.
v. Therapy with MSCs is not a readily available resource, especially in developing countries.
vi. High cost, insurance hurdles, and no standardized treatment protocol are issues of concern. The cost of MSC therapy is extremely variable (between $5000 and $50,000) and depends on the type of stem cells, laboratory location (for extraction), patient location (for infusion), and proliferation character of stem cells. To add to the complexity of the situation, in the United States, Medicare does not cover MSC therapy.
vii. In addition, MSC therapy is technically complex, requiring highly specialized staff and equipment.

Lab Medicine 2021;52;24–35 27
DOI: 10.1093/labmed/lmaa049

Table 1. List of Registered Cell-Based Clinical Trials for Treating COVID-19 Worldwide

ClinicalTrials.gov Identifier	Title	Study Characteristics	Primary Outcome Measures	Responsible Party
United States				
NCT04299152	Clinical Application of Stem Cell Educator Therapy for the Treatment of Viral Inflammation Caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	—Interventional study —Estimated participants: 20 —Study design: parallel	Determine the number of patients with COVID-19 who were unable to complete SCE therapy (time frame: 4 weeks)	Tianhe Stem Cell Biotechnologies Inc., China
NCT04348435	A Randomized, Double-Blind, Placebo-Controlled Clinical Trial to Determine the Safety and Efficacy of Hope Biosciences Allogeneic Mesenchymal Stem Cell Therapy (HB-adMSCs) to Provide Protection Against COVID-19	—Interventional study —Estimated participants: 100 —Study design: parallel	—Incidence of hospitalization for COVID-19 (time frame: week 0 through week 26 [end of study]) —Incidence of symptoms associated with COVID-19 (time frame: week 0 through week 26 [end of study])	Hope Biosciences Stem Cell Research Foundation, Sugar Land, TX
NCT04355728	Umbilical Cord-derived Mesenchymal Stem Cells for COVID-19 Patients with Acute Respiratory Distress Syndrome (ARDS)	—Interventional study —Estimated participants: 12 —Study design: parallel	—Incidence of prespecified infusion-associated AEs (time frame: day 5) —Incidence of SAEs (time frame: 90 days)	Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL
NCT04345601	Single Donor Banked Bone Marrow Mesenchymal Stromal Cells for the Treatment of SARS-CoV-2 Induced Acute Respiratory Failure: A Pilot Study	—Interventional study —Estimated participants: 30 —Intervention model: single group	—Incidence of unexpected AEs (time frame: 28 days post—cell infusion) —Improved oxygen saturations ≥ 93% (time frame: within 7 days of cell infusion)	Houston Methodist Hospital, Houston, TX
China				
ChiCTR2000031319	Safety and Efficacy Study of Allogeneic Human Dental Pulp Mesenchymal Stem Cells to Treat Severe Pneumonia of COVID-19: a Single-center, Prospective, Randomised Clinical Trial	—Interventional study —Estimated participants: 20 —Study design: parallel	…	Center for Regenerative Medicine, Renmin Hospital of Wuhan University, China
ChiCTR2000031735	Clinical study for natural killer (NK) cells from umbilical cord blood in the treatment of viral pneumonia include novel coronavirus pneumonia (COVID-19)	—Interventional study —Estimated participants: 20 —Study design: parallel	Monitoring of AEs within 24 hours after infusion (including infusion-related events and SAEs associated with nonprimary disease)	Huzhou Central Hospital, Zhejiang, China
ChiCTR2000031139	Safety and Effectiveness of Human embryonic stem cell-derived M cells (CAStem) for Pulmonary Fibrosis Correlated with novel coronavirus pneumonia (COVID-19)	—Interventional study —Estimated participants: 20 —Study design: sequential	—Pulmonary function evaluation —Changes in blood gas analysis —Evaluation of activity —Evaluation of dyspnea	Wuhan Jinyintan Hospital (Wuhan Infectious Diseases Hospital), Wuhan, Hubei, China
ChiCTR2000030509	Clinical Study of NK Cells in the Treatment of Novel Coronavirus Pneumonia (COVID-19)	—Interventional study —Estimated participants: 40 —Study design: parallel	Time and rate of novel coronavirus become negative	The First Hospital of Harbin Medical University, Harbin, Heilongjiang, China
ChiCTR2000030329	Clinical trial for umbilical cord blood CIK and NK cells in the treatment of mild and general patients infected with novel coronavirus pneumonia (COVID-19)	—Interventional study —Estimated participants: 90 —Study design: parallel	—Status of immune function —Nucleic acid test is negative —Length of stay in hospital	The Second Affiliated Hospital of Xi'an Medical University, Xi'an, Shaanxi, China
ChiCTR2000030224	Clinical study of mesenchymal stem cells in treating severe novel coronavirus pneumonia (COVID-19)	—Interventional study —Estimated participants: 32 —Study design: parallel	—Inflammatory biomarkers —Blood routine —Temperature —Lesions of lung seen in CT scan	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

Table 1. Continued

ClinicalTrials.gov Identifier	Title	Study Characteristics	Primary Outcome Measures	Responsible Party
ChiCTR2000030173	Key techniques of umbilical cord mesenchymal stem cells for the treatment of novel coronavirus pneumonia (COVID-19) and clinical application demonstration	—Interventional study —Estimated participants: 60 —Study design: parallel	—Pulmonary function —Novel coronavirus pneumonic nucleic acid test	Nanhua Hospital, affiliated with Nanhua University, Hengyang, Hu'nan, China
ChiCTR2000030088	Umbilical cord Wharton's Jelly derived mesenchymal stem cells in the treatment of severe novel coronavirus pneumonia (COVID-19)	—Interventional study —Estimated participants: 40 —Study design: parallel	—Nucleic acid test for novel coronavirus is negative —CT scan of ground glass shadow disappeared	The Sixth Medical Center of PLA General Hospital, Beijing, China
ChiCTR2000030020	The clinical application and basic research related to mesenchymal stem cells to treat novel coronavirus pneumonia (COVID-19)	—Interventional study —Estimated participants: 40 —Study design: sequential	—Coronavirus nucleic acid markers have negative rate —Inflammation (per chest CT) —Symptoms improved after 4 treatments	Second Hospital of University of South China, Hengyang, China
ChiCTR2000029816	Clinical Study of Cord Blood Mesenchymal Stem Cells in the Treatment of Acute Novel Coronavirus Pneumonia (COVID-19)	—Interventional study —Estimated participants: 60 —Study design: parallel	Time to disease recovery	Guangzhou Reborn Health Management Consultation Co, Ltd, Guangzhou, Guangdong, China
ChiCTR2000029812	Clinical Study for Umbilical Cord Blood Mononuclear Cells in the Treatment of Acute Novel Coronavirus Pneumonia (NCP)	—Interventional study —Estimated participants: 60 —Study design: parallel	Time to disease recovery	Guangzhou Reborn Health Management Consultation Co, Ltd, Guangzhou, Guangdong, China
ChiCTR2000029606	Clinical Study for Human Menstrual Blood-derived Stem Cells in the Treatment of Acute Novel Coronavirus Pneumonia (COVID-19)	—Interventional study —Estimated participants: 63 —Study design: parallel	Mortality in patients	The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China
ChiCTR2000029580	Severe novel coronavirus pneumonia (COVID-19) patients treated with ruxolitinib in combination with mesenchymal stem cells: a prospective, single blind, randomized controlled clinical trial	—Interventional study —Estimated participants: 70 —Study design: parallel	Safety	Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
ChiCTR2000029572	Safety and efficacy of umbilical cord blood mononuclear cells in the treatment of severe and critically novel coronavirus pneumonia (COVID-19): a randomized controlled clinical trial	—Interventionalstudy —Estimated participants: 30 —Study design: parallel	PSI	Xiangyang First People's Hospital, Xiangyang, Hubei, China
ChiCTR2000029569	Safety and efficacy of umbilical cord blood mononuclear cells conditioned medium in the treatment of severe and critically novel coronavirus pneumonia (COVID-19): a randomized controlled trial	—Interventional study —Estimated participants: 30 —Study design: parallel	PSI	Xiangyang First People's Hospital, Xiangyang, Hubei, China
CTR2000030116	Safety and effectiveness of human umbilical cord mesenchymal stem cells in the treatment of acute respiratory distress syndrome of severe novel coronavirus pneumonia (COVID-19)	Interventional study —Estimated participants: 16 —Study design: dose comparison	Time to leave ventilator on day 28 after receiving MSC infusion	The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China

Table 1. Continued

ClinicalTrials.gov Identifier	Title	Study Characteristics	Primary Outcome Measures	Responsible Party
ChiCTR2000030484	HUMSCs and Exosomes Treating Patients with Lung Injury following Novel Coronavirus Pneumonia (COVID-19)	—Interventional study —Estimated participants: 90 —Study design: parallel	—PaO_2/FiO_2 or respiratory rate (without oxygen) —Frequency of respiratory exacerbation —Observe physical signs and symptoms and record clinical recovery time —Number and range of lesions indicated by CT and X-ray of lung —Time for cough to become mild or absent —Time for dyspnea to become mild or no dyspnea —Frequency of oxygen inhalation or noninvasive ventilation, frequency of mechanical ventilation —Inflammatory cytokines (eg, CRP/PCT/SAA) —Frequency of SAE	Hubei Shiyan Taihe Hospital, Shiyan, Hubei, China
ChiCTR2000030866	Open-label, observational study of human umbilical cord derived mesenchymal stem cells in the treatment of severe and critical COVID-19.	Observational study —Estimated participants: 30 Study design: single arm	-Oxygenation index (PaO_2/FiO_2) —Conversion rate from serious to critical patients —Conversion rate and conversion time from critical to serious patients —Mortality in serious and critical patients	Changsha First Hospital, Changsha, Hu'nan, China
ChiCTR2000030835	Clinical study on the efficacy of Mesenchymal stem cells (MSC) in the treatment of severe novel coronavirus pneumonia (COVID-19)	—Interventional study —Estimated participants: 20 —Study design:single arm	NA	The First Affiliated Hospital of Xinxiang Medical University, Xinxiang, He'nan, China
ChiCTR2000030138	Clinical Trial for Human Mesenchymal Stem Cells in the Treatment of Severe Novel Coronavirus Pneumonia (COVID-19)	—Interventional study —Estimated participants: 60 —Study design: parallel	Clinical index	Chinese PLA General Hospital, Haidian Distract, Beijing, China
NCT04302519	Clinical Study of Novel Coronavirus Induced Severe Pneumonia Treated by Dental Pulp Mesenchymal Stem Cells	—Interventional study —Estimated participants: 24 Study design: single group	Disappear time of ground-glass shadow in lungs (time frame: 14 days)	CAR-T (Shanghai) Biotechnology Co, Ltd, Shanghai, China
NCT04288102	Multicenter, Randomized, Double-blind, Placebo-controlled Study Evaluating the Efficacy and Safety of Human Mesenchymal Stem Cells in Combination with Standard Therapy in the Treatment of COVID-19 Patients With Severe Convalescence	—Interventional study —Estimated participants: 90 —Study design: parallel	Size of lesion area and severity of pulmonary fibrosis by chest CT (time frame: at baseline, day 6, day 10, day 14, day 28, day 90]	Maternal and Child Hospital of Hubei Province, Wuhan, Hubei, China, and Wuhan Huoshenshan Hospital, Wuhan, Hubei, China

Table 1. Continued

ClinicalTrials.gov Identifier	Title	Study Characteristics	Primary Outcome Measures	Responsible Party
NCT04273646	Clinical Study of Human Umbilical Cord Mesenchymal Stem Cells in the Treatment of Severe COVID-19	—Interventional study —Estimated participants: 48 —Study design: parallel	—Pneumonia severity index (time frame: from baseline to 12 weeks after treatment) —Oxygenation index (PaO_2/FiO_2; time frame: from baseline to 12 weeks after treatment)	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
NCT04252118	Safety and Efficiency of Mesenchymal Stem Cell in Treating Pneumonia Patients Infected With COVID-19	—Interventional study —Estimated participants: 20 —Study design: parallel	—Size of lesion area by chest radiograph or CT (time frame: at baseline, day 3, day 6, day 10, day 14, day 21, day 28) —Side effects in MSC treatment group (time frame: at baseline, day 3, day 6, day 10, day 14, day 21, day 28, day 90, day 180)	Beijing 302 Military Hospital of China, Beijing, China
NCT04269525	Clinical Research Regarding the Availability and Safety of UC-MSCs Treatment for Serious Pneumonia and Critical Pneumonia Caused by the 2019-nCOV Infection	—Interventional study —Estimated participants: 10 —Intervention model: single group	Oxygenation index (time frame: on day 14 after enrollment)	Zhongnan Hospital, Wuhan, Hubei, China
NCT04276987	A Pilot Clinical Study on Aerosol Inhalation of the Exosomes Derived From Allogenic Adipose Mesenchymal Stem Cells in the Treatment of Severe Patients With Novel Coronavirus Pneumonia	—Interventional study —Estimated participants: 30 —Intervention model: single group	—Time to clinical improvement (time frame: up to 28 days) —AE and SAE (time frame: up to 28 days)	Ruijin Hospital, Wuhan, China
NCT04346368	Safety and Efficacy of Intravenous Infusion of Bone Marrow-Derived Mesenchymal Stem Cells in Severe Patients with Coronavirus Disease 2019 (COVID-19): A Phase 1/2 Randomized Controlled Trial	—Interventional study —Estimated participants: 20 —Intervention model: Single group	—AEs in BM-MSC treatment group (time Frame: baseline–6 months) —Changes in oxygenation index (PaO_2/FiO_2; time frame: baseline, 6hours, day 1, day 3, week 1, week 2, week 4, month 6)	Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong, China
NCT04339660	Clinical Research of Human Mesenchymal Stem Cells in the Treatment of COVID-19 Pneumonia	—Interventional study —Estimated participants: 30 —Study design: parallel	—Observe immune function (TNF-α, IL-1β, IL-6, TGF-β, IL-8, PCT, CRP; time frame: within 4 weeks) —Monitor blood oxygen saturation (time frame: within 4 weeks)	Puren Hospital, affiliated with Wuhan University of Science and Technology, Wuhan, Hubei, China
NCT04331613 Brazil	Safety and Efficacy Study of Human Embryonic Stem Cells Derived M Cells (CAStem) for the Treatment of Severe COVID-19 Associated With or Without Acute Respiratory Distress Syndrome (ARDS)	—Interventional study —Estimated participants: 09 —Study design: single group	—AE and SAE (time frame: within 28 days after treatment) —Changes in lung imaging examinations (time frame: within 28 days after treatment)	Beijing YouAn Hospital, Capital Medical University Beijing, Beijing, China,
NCT04315987 Jordan	Exploratory Clinical Study to Assess the Efficacy of NestCell Mesenchymal Stem Cell to Treat Patients with Severe COVID-19 Pneumonia	—Interventional study —Estimated participants :66 —Study design: single group	Change in clinical condition (time frame: 10 days)	Hospital Vera Cruz Campina Grande, São Paulo, Brazil

Table 1. Continued

ClinicalTrials.gov Identifier	Title	Study Characteristics	Primary Outcome Measures	Responsible Party
NCT04313322	Treatment of COVID-19 Patients Using Wharton's Jelly-Mesenchymal Stem Cells	—Interventional study —Estimated participants: 5 —Intervention model: single group	—Improvement of clinical symptoms (time frame: 3 weeks) —AEs measured by chest radiograph/CT scan (time frame: 3 weeks) —RT-PCR results of viral RNA turning negative (time frame: 3 weeks)	Stem Cells Arabia, Amman, Jordan
France				
NCT04333368	Cell Therapy Using Umbilical Cord-derived Mesenchymal Stromal Cells in SARS-CoV-2-related ARDS	—Interventional study —Estimated participants: 60 —Intervention model: single group	Respiratory efficacy evaluated by increase in PaO_2/FiO2 ratio from baseline to day 7 in experimental group compared with placebo group	Hôpital Pitié-Salpêtrière—APHP, Paris, France, and Hôpital Européen Georges Pompidou—APHP, Paris, France
United Kingdom				
NCT04349540	A Prospective Non-Interventional Study to Evaluate the Role of Immune and Inflammatory Response in Recipients of Allogeneic Haematopoietic Stem Cell Transplantation (SCT) Affected by Severe COVID19 Infection	—Observational study —Estimated participants: 40 —Intervention model: case-only	…	Great Ormond Street Hospital, NHS Foundation Trust, London, United Kingdom
NCT03042143	Repair of Acute Respiratory Distress Syndrome by Stromal Cell Administration (REALIST): An Open Label Dose Escalation Phase 1 Trial Followed by a Randomized, Double-blind, Placebo-controlled Phase 2 Trial (COVID-19)	—Interventional study —Estimated participants: 75 —Study design: parallel	—Oxygenation index (time frame: day 7) —Incidence of SAEs (time frame: 28 days)	Belfast Health and Social Care Trust, Royal Hospitals Belfast, Northern Ireland, United Kingdom
Spain				
NCT04348461	Two-treatment, Randomized, Controlled, Multicenter Clinical Trial to Assess the Safety and Efficacy of Intravenous Administration of Expanded Allogeneic Adipose Tissue Adult Mesenchymal Stromal Cells in Critically Ill Patients COVID-19	—Interventional study —Estimated participants: 100 —Study design: parallel	—Efficacy of administration of allogeneic MSCs derived from AT assessed by survival rate (time frame: 28 days) —Safety of administration of allogeneic MSCs derived from AT assessed by AE rate (time frame: 6 months)	Instituto de Investigación, Sanitaria de la Fundación Jiménez Díaz, Madrid, Spain
Denmark				
NCT04341610	Allogeneic Adipose Tissue Derived Mesenchymal Stromal Cell Therapy for Treating Patients with Severe Respiratory COVID-19. A Danish, Double-blind, Randomized Placebo-controlled Study	—Interventional study —Estimated participants: 40 —Study design: parallel	Changes in clinical critical treatment index (time frame: day 7 from randomization)	Department of Cardiology, The Heart Centre, University Hospital Rigshospitalet, Copenhagen, Denmark

SCE, stem cell educator; AE, adverse event; SAE, serious adverse event; CT, computed tomography; PSI, patient safety indicators; MSCs, mesenchymal stem cells; PaO2, partial pressure of oxygen; FiO2, fraction of inspired oxygen; PCT, procalcitonin; SAA, serum amyloid A; CRP, C-reactive protein; BM-MSC, bone marrow mesenchymal stem cells; RT-PCR, real-time polymerase chain reaction; AT, adipose tissue

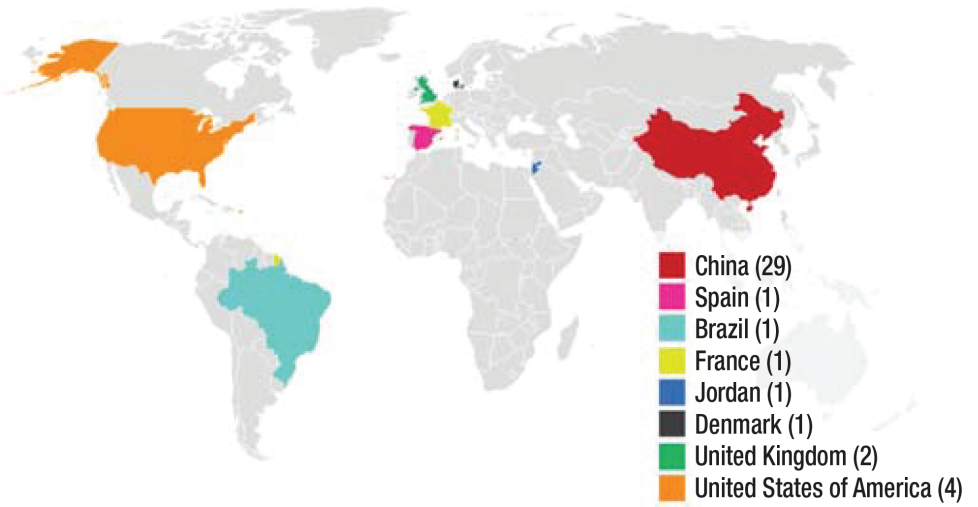


Figure 1

Number of studies currently enrolled regarding MSCs in COVID-19 by country (as of April 22, 2020).

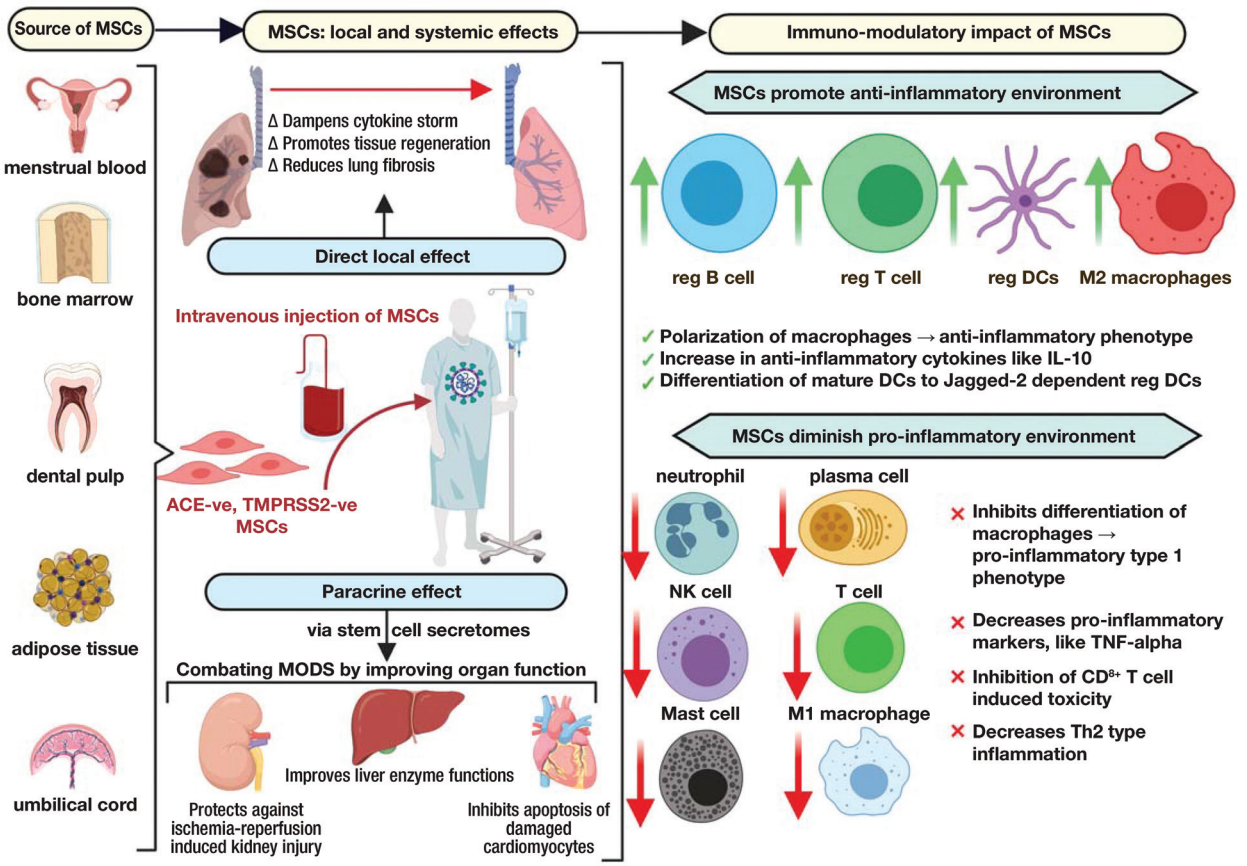


Figure 2

Pictorial description of the source of stem cells, and impact of mesenchymal stem cells.

Lab Medicine 2021;52;24–35
DOI: 10.1093/labmed/lmaa049

viii. Scalability because of these technical challenges may lead to a high discrepancy in the supply-demand chain.

ix. There could be immediate adverse effects from MSC infusion; transfusion reactions such as allergies, anaphylaxis, serum sickness, delayed hypersensitivity reactions, and secondary bacteremia. On a positive note, Leng et al[11] did not note any acute infusion-related issues, allergic reactions, or delayed hypersensitivity or secondary infections in their cohort of patients. Researchers have found that MSCs are ACE-2 negative and TMPRSS2 negative and are thereby unlikely to become infected by SARS-CoV-2.

Conclusion

During this unprecedented healthcare crisis, researchers and clinicians across the globe are working relentlessly to identify the best strategies and treatment for patients with COVID-19. Although MSCs are a potentially promising therapy, they are still in the early stages of development for COVID-19 treatment. Further data from ongoing clinical trials across the world will help clarify their potential utility in battling the COVID-19 pandemic. **LM**

Acknowledgments

The article does not contain the participation of any human being or animal. All authors have seen the article and agree to the content and data. All the authors played a significant role in the article.

References

1. Sahu KK, Kumar R. Current perspective on pandemic of COVID-19 in the United States. *J Family Med Prim Care.* 2020;9(4):1784.
2. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature.* 2020;579(7798):265–269.
3. Sahu KK, Kumar R. Preventive and treatment strategies of COVID-19: from community to clinical trials. *J Family Med Prim Care*. 2020;9(5):2149.
4. Coronavirus update (Live): 6,102,522 cases and 369,127 deaths from COVID-19 virus pandemic – worldometer website updated, June 03, 2020. https://www.worldometers.info/coronavirus/. Accessed June 03 2020.
5. Amanat F, Krammer F. SARS-CoV-2 vaccines: status report. *Immunity.* 2020;52(4):583–589.
6. Douedi S, Miskoff J. Novel coronavirus 2019 (COVID-19): a case report and review of treatments. *Medicine (Baltimore).* 2020;99(19):e20207.
7. Qu W, Wang Z, Hare JM, et al. Cell-based therapy to reduce mortality from COVID-19: Systematic review and meta-analysis of human studies on acute respiratory distress syndrome [published online ahead of print May 29, 2020]. *Stem Cells Transl Med*. 2020.
8. Weiss ARR, Dahlke MH. Immunomodulation by Mesenchymal Stem Cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. *Front Immunol*. 2019;10:1191.
9. Sahu KK, Jindal V, Siddiqui AD, Cerny J, Gerber JM. Convalescent plasma therapy: a passive therapy for an aggressive COVID-19 [published online ahead of print May 21, 2020]. *J Med Virol*. 2020; doi:10.1002/jmv.26047.
10. Sahu KK, Lal A, Mishra AK. Latest updates on COVID-2019: a changing paradigm shift. *J Med Virol*. 2020;92(6):533–535.
11. Leng Z, Zhu R, Hou W, et al. Transplantation of ACE2- mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging Dis.* 2020;11(2):216–228.
12. Golchin A, Seyedjafari E, Ardeshirylajimi A. Mesenchymal stem cell therapy for COVID-19: present or future. *Stem Cell Rev Rep*. 2020;16(3):427–433
13. Metcalfe SM. Mesenchymal stem cells and management of COVID-19 pneumonia. *Med Drug Discov*. 2020;5:100019.
14. Liu S, Peng D, Qiu H, Yang K, Fu Z, Zou L. Mesenchymal stem cells as a potential therapy for COVID-19. *Stem Cell Res Ther.* 2020;11(1):169.
15. Parekkadan B, Milwid JM. Mesenchymal stem cells as therapeutics. *Annu Rev Biomed Eng*. 2010;12:87–117.
16. Atluri S, Manchikanti L, Hirsch JA. Expanded umbilical cord mesenchymal stem cells (UC-MSCs) as a therapeutic strategy in managing critically ill COVID-19 patients: the case for compassionate use. *Pain Physician.* 2020;23(2):E71–E83.
17. Nitkin CR, Rajasingh J, Pisano C, Besner GE, Thébaud B, Sampath V. Stem cell therapy for preventing neonatal diseases in the 21st century: Current understanding and challenges. *Pediatr Res*. 2020;87(2):265–276.
18. Öztürk S, Elçin AE, Elçin YM. Mesenchymal stem cells for coronavirus (COVID-19)-induced pneumonia: revisiting the paracrine hypothesis with new hopes? *Aging Dis*. 2020;11(3):477.
19. Shetty AK. Mesenchymal stem cell infusion shows promise for combating coronavirus (COVID-19)- induced pneumonia. *Aging Dis.* 2020;11(2):462–464.
20. Cuiffo BG, Karnoub AE. Mesenchymal stem cells in tumor development: emerging roles and concepts. *Cell Adh Migr.* 2012;6(3):220–230.
21. Hayal TB, Kıratlı B, Şişli HB, Şahin F, Doğan A. Mesenchymal stem cells as regulators of carcinogenesis. *Adv Exp Med Biol*. 2019;1144:147–166.
22. Marks PW, Witten CM, Califf RM. Clarifying stem-cell therapy's benefits and risks. *N Engl J Med.* 2017;376(11):1007–1009.
23. FDA warns about stem cell therapies | FDA. U.S. Food and Drug Administration website, Updated September 03, 2019. https://www.fda.gov/consumers/consumer-updates/fda-warns-about-stem-cell-therapies. Accessed June 06, 2020.
24. Approved cellular and gene therapy products | FDA. U.S. Food and Drug Administration website, Updated September 03, 2019. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products. Accessed June 06, 2020.
25. Hope Biosciences. Hope Biosciences gets FDA nod for phase 2 COVID-19 stem cell therapy trial. Updated April 14, 2020. https://pharmaceutical-business-review.com/news/hope-biosciences-covid-19-stem-cell/. Accessed June 06, 2020.
26. Slater H. *FDA accepts IND for NK cell therapy CYNK-001 to treat patients with COVID-19*. Updated April 03, 2020. Accessed June 06, 2020.

27. Chen Y, Chan VSF, Zheng B, et al. A novel subset of putative stem/progenitor CD34+ Oct-4 + cells is the major target for SARS coronavirus in human lung. *J Exp Med.* 2007;204(11):2529–2536.
28. Jamilloux Y, Henry T, Belot A, et al. Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. *Autoimmun Rev.* 2020;19(7):102567.
29. Sahu KK, Siddiqui AD. From Hematologist's desk: the effect of COVID-19 on the blood system [published online ahead of print May 1, 2020]. *Am J Hematol*. 2020;doi:10.1002/ajh.25849.
30. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497–506.
31. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest.* 2020;130(5):2620–2629.
32. Kotton DN, Morrisey EE. Lung regeneration: mechanisms, applications and emerging stem cell populations. *Nat Med.* 2014;20(8):822–832.
33. Liu Q, Zhang C, Sun A, Zheng Y, Wang L, Cao X. Tumor-educated CD11b high Ia low regulatory dendritic cells suppress T cell response through arginase I. *J Immunol*. 2009;182(10):6207–6216.
34. Zhang B, Liu R, Shi D, et al. Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population. *Blood.* 2009;113(1):46–57.
35. Liu X, Qu X, Chen Y, et al. Mesenchymal stem/stromal cells induce the generation of novel IL-10–dependent regulatory dendritic cells by SOCS3 activation. *J Immunol*. 2012;189(3):1182–1192.
36. Zhao RC. Stem cell-based therapy for coronavirus disease 2019. *Stem Cells Dev.* 2020;29(11):679–681.
37. Rebelatto CK, Aguiar AM, Moretão MP, et al. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. *Exp Biol Med (Maywood).* 2008;233(7):901–913.
38. Rogers CJ, Harman RJ, Bunnell BA, et al. Rationale for the clinical use of adipose-derived mesenchymal stem cells for COVID-19 patients. *J Transl Med.* 2020;18(1):203.
39. Gentile P, Sterodimas A. Adipose-derived stromal stem cells (ASCs) as a new regenerative immediate therapy combating coronavirus (COVID-19)-induced pneumonia. *Expert Opin Biol Ther*. 2020;20(7):711–716.
40. Atluri S, Manchikanti L, Hirsch JA. Expanded umbilical cord mesenchymal stem cells (UC-MSCs) as a therapeutic strategy in managing critically ill COVID-19 patients: the case for compassionate use. *Pain Physician*. 2020;23(2):E71–E83.
41. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells.* 2006;24(5):1294–1301.
42. Mossel EC, Wang J, Jeffers S, et al. SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells. *Virology.* 2008;372(1):127–135.
43. Sims AC, Burkett SE, Yount B, Pickles RJ. SARS-CoV replication and pathogenesis in an in vitro model of the human conducting airway epithelium. *Virus Res.* 2008;133(1):33–44.
44. Qian Z, Travanty EA, Oko L, et al. Innate immune response of human alveolar type II cells infected with severe acute respiratory syndrome-coronavirus. *Am J Respir Cell Mol Biol.* 2013;48(6):742–748.
45. Wichmann D, Sperhake JP, Lütgehetmann M, et al. Autopsy findings and venous thromboembolism in patients with COVID-19 [published online ahead of print May 6, 2020]. *Ann Intern Med*. 2020;M20-2003.
46. Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem cell transplantation: the lung barrier. *Transplant Proc.* 2007;39(2):573–576.
47. Chen J, Hu C, Chen L, et al. Clinical study of mesenchymal stem cell treatment for acute respiratory distress syndrome induced by epidemic influenza A (H7N9) infection: a hint for COVID-19 treatment. *Engineering*. 2020.
48. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med.* 2020;8(5):475–481.
49. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med.* 2020;8(4):e21.
50. Sahu KK, Siddiqui AD, Cerny J. Managing sickle cell patients with COVID-19 infection: the need to pool our collective experience [published online ahead of print May 23, 2020]. *Br J Haematol*. 2020.
51. Sahu KK, Siddiqui AD, Cerny J. COVID-19 pandemic and impact on hematopoietic stem cell transplantation [published online ahead of print May 4, 2020]. *Bone Marrow Transplant*. 2020;1–3.
52. Jindal V, Sahu KK, Gaikazian S, Siddiqui AD, Jaiyesimi I. Cancer treatment during COVID-19 pandemic. *Med Oncol.* 2020;37(7):58.
53. Hayes M, Curley G, Ansari B, Laffey JG. Clinical review: stem cell therapies for acute lung injury/acute respiratory distress syndrome—hope or hype? *Crit Care.* 2012;16(2):205.
54. Yu F, Jia R, Tang Y, Liu J, Wei B. SARS-CoV-2 infection and stem cells: Interaction and intervention [published online ahead of print June 1, 2020]. *Stem Cell Res*. 2020;46:101859.
55. **中国临床试验注册中心 - 世界卫生组织国际临床试验注册平台一级注册机构**. Updated May 15, 2020. Available from: http://www.chictr.org.cn/index.aspx. Accessed June 06, 2020
56. Home - ClinicalTrials.gov. Updated May 20, 2020. Available from: https://clinicaltrials.gov/. Accessed June 06, 2020
57. Musiał-Wysocka A, Kot M, Majka M. The pros and cons of mesenchymal stem cell-based therapies. *Cell Transplant.* 2019;28(7):801–812.
58. Aliotta JM, Keaney P, Passero M, et al. Bone marrow production of lung cells: the impact of G-CSF, cardiotoxin, graded doses of irradiation, and subpopulation phenotype. *Exp Hematol.* 2006;34(2):230–241.
59. Glowacka I, Bertram S, Herzog P, et al. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. *J Virol.* 2010;84(2):1198–1205.
60. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med.* 2005;11(8):875–879.
61. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol.* 2004;203(2):631–637.

Quantitative Assessment of the Effects of *IL-1ß* -511 C>T Variant on Breast Cancer Risk: An Updated Meta-Analysis of 3331 Cases and 3609 Controls

Mahdiyeh Harati-Sadegh, PhD,[1] Milad Mohammadoo-Khorasani, PhD,[2] Saman Sargazi, PhD,[3] Ramin Saravani, PhD,[3,4] Sheida Shahraki, MSc,[3] Ebrahim Eskandari, PhD[5*]*

Laboratory Medicine 2021;52:36-46

DOI: 10.1093/labmed/lmaa055

ABSTRACT

Objective: Growing evidence suggests that *IL-1*β -511C>T, as a functional variant, affects the risk of developing breast cancer (BC); however, the results have not been conclusive. This meta-analysis was conducted to estimate the link between this variant and BC risk.

Methods: We retrieved available publications on *IL-1*β -511C>T polymorphism by conducting a comprehensive literature search on the Web of Science, MEDLINE, PubMed, Scopus, and Google scholar databases (last search on February 25, 2020).

Results: The overall analysis indicates that *IL-1*β -511C>T polymorphism conferred an increased risk of BC under a recessive TT vs CT+CC model by 1.14-fold and showed protection against BC under an overdominant CT vs TT+CC genetic contrast model (odds ratio = 0.84). Stratified analysis based on ethnicity revealed the protective effect of this single-nucleotide polymorphism against BC risk in Caucasian patients.

Conclusion: Our data results provide a proof of concept for the association of *IL-1*β -511C>T with BC risk. Larger, well-designed population-based studies are needed to confirm these findings.

Keywords: breast cancer, IL-1β, meta-analysis, polymorphism

Breast cancer (BC) is one of the most common malignancies in women worldwide, and it remains a major global health concern with a significant morbidity and mortality.[1] According to the 2018 Global Cancer Statistics in 185 countries, 2,088,849 individuals were diagnosed with BC, and 626,679 related deaths were reported as the consequence of the disease.[2] Research has shown that BC is a multifactorial disease and that genetic and environmental factors play important roles in its pathogenesis.[3] Single-nucleotide polymorphisms (SNPs) have been suggested to affect the risk of developing BC in individuals.[4,5]

Interleukin-1 (IL-1) is a proinflammatory cytokine (proteins that are released by immune cells, epithelial cells, and so on) that plays important roles in the development of inflammation-linked malignancies.[6-9] It is released from white blood cells, macrophages, epithelial cells, and tumor cells (ie, tumor-associated fibroblasts),[10] and modulates the host response to inflammation, tissue injury, and cell proliferation.[11,12] The IL-1 family includes 2 ligands (IL-1α and IL-1β) and 3 receptors (IL-1Ra = receptor antagonist; IL-1RI and IL-1RII).[13] The ligand IL-1β regulates

Abbreviations:

BC, breast cancer; SNP, single-nucleotide polymorphism; IL-1, interleukin-1; OR, odds ratio; QA, quality assessment; CI, confidence interval; TSA, trial sequential analysis; RIS, required information size; FPRP, false-positive report probability; AA, African American; As, Asian; Ca, Caucasian; EA, European American; HWE, Hardy-Weinberg equilibrium; PCR-RFLP, polymerase chain reaction- restriction fragment length polymorphism

[1]Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran, [2]Department of Clinical Biochemistry, School of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran, [3]Cellular and Molecular Research Center, Resistant Tuberculosis Institute and [4]Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran, [5]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

*To whom correspondence should be addressed.
sgz.biomed@gmail.com

the proliferation of breast cancer cells through estrogen production by steroid-catalyzing enzymes.[14,15] Expression of IL-1 in primary tumors of patients with breast cancer has been shown to predict the risk of developing bone metastasis.[16]

The *IL-1*β gene (OMIM: 147720) is located on chromosome 2q14.2.[17] Genetic variants located in the *IL-1*β gene may affect the expression of the *IL-1*β gene.[18] The *IL-1*β -511C>T (rs16944) variant is located in the promoter region of the *IL-1*β gene. The minor allele frequency of this variant is 0.49 in the global population, according to 1000 Genome Project Phase 3[19] (http://www.internationalgenome.org/category/population). Polymorphism in IL-*1*β -511C>T has been associated with various types of solid cancers, including breast cancer.[20] Several case-control studies in patients with different ethnicities have suggested that the *IL-1*β -511C>T polymorphism may affect the risk of BC. However, the results of these single studies have been inconsistent, possibly because of ethnicity-related differences in genetic makeup or limited sample size. Because meta-analyses are useful tools that can improve the statistical power of individual association studies and can resolve the discrepancies among the results of independent studies,[21] we conducted a meta-analysis to assess the association between the *IL-1*β -511C>T polymorphism and breast cancer risk in diverse inheritance models.

Materials and Methods

Literature Search

We searched the Web of Knowledge, MEDLINE, PubMed, Scopus, and Google Scholar databases (the last search was conducted February 25, 2020) for all articles including the following keywords: "Interleukin-1 or Interleukin-1beta or IL-1beta or IL-1b or Interleukin-1b or Interleukin-1b-511 or IL-1b-511 or IL-1beta-511 or rs16944" and "polymorphism or variant or mutation or SNP" and "breast cancer or breast adenocarcinoma or breast neoplasm or breast tumor." Additional eligible studies were identified by manually searching retrieved articles. The inclusion criteria for the current meta-analysis were as follows: (i) original case-control study exploring the link between *IL-1*β -511C>T polymorphism and BC susceptibility, and (ii) studies containing original data for odds ratio (OR) calculation in both studied groups. Studies not published in English, cohort studies, case reports, meta-analyses, review articles, conference abstracts, and duplicated data were excluded. Two separate case-control studies reported in the same article were considered as 2 independent studies. Eligible studies were published from 2004 to 2020.

Data Extraction and Quality Assessment

As independent investigators, SS and MHS extracted the data from articles and recorded the first author name, year of publication, country, patients' ethnicity, numbers of patients in the case and control groups, type of genotyping method, and the allelic and genotypic distribution of *IL-1*β -511C>T in participants. A third investigator (MMK) rechecked the results for possible inconsistencies. The quality assessment (QA) of the included studies was performed as described previously,[22] and scores are summarized in **Table 1**. Studies with a score ≤9 were considered low quality, whereas those scoring >9 were considered high quality.

Statistical Analysis

We used the MetaGenyo web tool[23] and Stata v.12 software to carry out the statistical analysis. The χ^2 test was used to assess Hardy-Weinberg equilibrium violations in the control groups. The strength of the link between the SNP and the BC risk was estimated by the calculation of ORs and 95% confidence intervals (CI). Five types of genetic contrast models were used in the meta-analysis: (i) an allelic model (T vs C), (ii) codominant models (CT vs CC and TT vs CC), (iii) a dominant model (CT+TT vs CC), (iv) a recessive model (TT vs CT+CC), and (v) an overdominant model (CT vs TT+CC). For heterogeneity analysis, the Mantel-Haenszel method was employed based on I^2 statistics. In the case of between-study heterogeneity (>50% as heterogeneity), a random-effects model was used. Otherwise, a fixed-effects model was applied. The Egger funnel plot asymmetry test was employed to determine publication bias. To measure the robustness of pooled ORs, a sensitivity analysis was conducted.

DOI: 10.1093/labmed/lmaa055

Table 1. Characteristics of All Studies Included in Meta-Analysis

Study	Genotyping Method	Country	Ethnicity	Patients	Control Patients	CC Patients	CT Patients	TT Patients	C Patients	T Patients	CC Control Patients	CT Control Patients	TT Control Patients	C Control Patients	T Control Patients	P_{HWE}[a]	Score
Smith et al[27]	ARMS-PCR	United Kingdom	Ca	141	261	60	67	14	187	95	87	135	39	309	213	.57	8
Hefler et al[28]	Pyrosequencing	Austria	Ca	269	227	124	114	31	362	176	88	111	28	287	167	.71	9
Balasubramanian et al[29] (2006)	TaqMan	United Kingdom	Ca	703	489	339	294	70	972	434	232	206	51	670	308	.71	13
Liu, Zhai, et al[30]	PCR-RFLP	China	As	270	631	94	170	101	291	249	197	305	129	699	563	.71	12
Kaarvatn et al[31]	TaqMan	Croatia	Ca	193	188	106	65	22	277	109	79	88	21	246	130	.71	10
Gong et al[32] part 1	Mass array	United States	EA	326	309	137	140	49	414	238	143	134	32	420	198	.94	11
Gong et al[32] part 2	Mass array	United States	AA	450	403	90	229	131	409	491	93	185	125	371	435	.38	11
Zuo et al[33]	Mass array	China	As	530	628	160	236	134	556	504	142	344	142	628	628	.09	12
Wang et al[34]	Mass array	China	As	298	287	90	132	76	312	284	68	163	56	299	275	.09	11
Al-Eitan et al[35]	PCR-RFLP	Jordan	As	151	186	56	65	30	177	125	74	84	28	232	140	.71	12

AA, African American; ARMS-PCR, Amplification refractory mutation system; As, Asian; Ca, Caucasian; EA, European American; HWE, Hardy-Weinberg equilibrium; PCR-RFLP, polymerase chain reaction- restriction fragment length polymorphism.

[a]P_{HWE}: *HWE P value for control patients was corrected for multiple testing by the false discovery rate method.*

DOI: 10.1093/labmed/lmaa055

Trial Sequential Analysis and False-Positive Report Probability Analyses

We applied trial sequential analysis (TSA) to increase the robustness of conclusions and minimize random errors caused by sparse data and repetitive testing.[24] We used TSA software version 0.9.5.10 (http://www.ctu.dk/tsa/) to calculate the required information size (RIS; meta-analysis sample size),[25] which was based on the assumption of a plausible relative risk of 10% with a low risk bias and a significance of 5% for type I errors and 20% for type II errors (power 80%). The TSA monitoring boundaries were generated according to the RIS and the risk for type I and type II errors. If the cumulative *Z* curve crossed a TSA monitoring boundary (red lines sloping inward) before the required information size was reached, the robustness of evidence may be confirmed and further trials were unnecessary. Otherwise, the evidence was inadequate to get a robust conclusion and it would be necessary to continue trials. The false-positive report probability (FPRP) values at different prior probability levels for all significant findings were assessed.[26] A FPRP value <0.2 represented a noteworthy association.

Results

Study Characteristics

A total of nine articles (10 separate case-control studies) involving 3331 patients with BC and 3609 control patients were included to assess the association between *IL-1*β -511C>T polymorphism and BC risk.[27-35] **Figure 1** shows a diagram of the search procedure. The characteristics of each included study are shown in **Table 1**.

Pooled Analysis

Table 2 summarizes the results of the pooled analysis. The pooled ORs from eligible studies revealed that the *IL-1*β -511C>T variant conferred an increased risk of BC under the recessive genetic comparison model (OR, 1.14; 95% CI, 1.01–1.30; *P* =.031; TT vs CT+CC; **Figure 2**). In contrast, this SNP was associated with protection against BC susceptibility under the overdominant CT vs TT+CC model (OR, 0.84; 95% CI, 0.72–0.98; *P* =.026). However, no significant association was found with regard to the *IL-1*β -511C>T variant and BC risk under the allelic, codominant homozygote, codominant heterozygote, and dominant contrasted models.

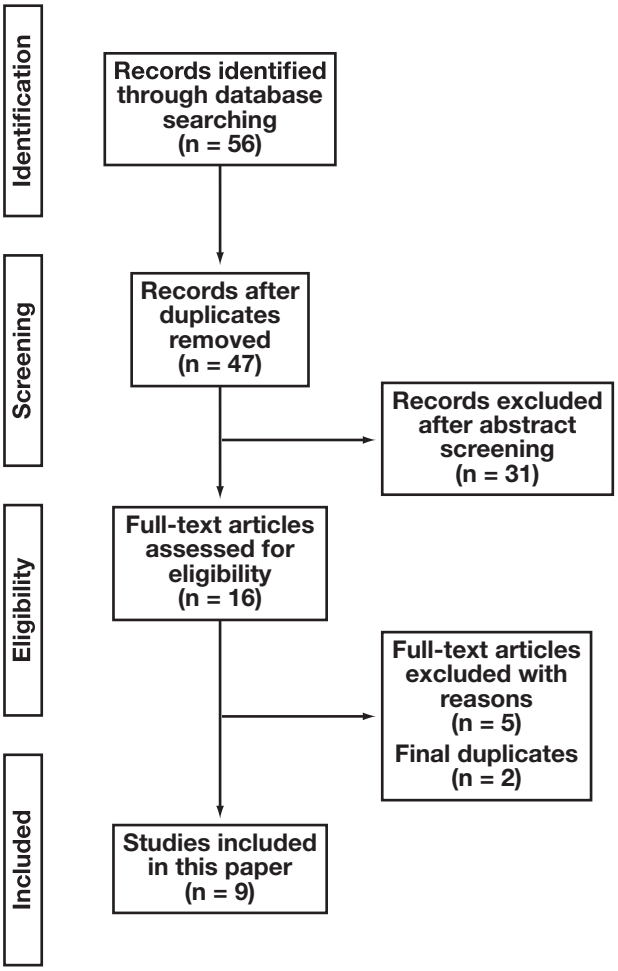


Figure 1

Flow diagram of study selection process.

Stratified Analysis by Ethnicity and Genotyping Method

In the stratification analysis by ethnicity, a significant association was found between the *IL-1*β -511C>T variant and a reduced risk of BC in Caucasian patients using the allelic (OR, 0.86; 95% CI, 0.76–0.97; *P* =.02), codominant CT vs CC (OR, 0.80; 95% CI, 0.68–0.95; *P* =.01), dominant (OR, 0.80; 95% CI, 0.68–0.95; *P* =.008), and

DOI: 10.1093/labmed/lmaa055

Table 2. Results of Association Test, Heterogeneity, and Publication Bias of the *IL-1β* -511 C>T Polymorphism on BC Risk

Genetic Model	Number of Studies	Association Test		Model	Heterogeneity Test		Eggers Funnel Plot Test
		OR (95% CI)	*P* Value		*P* Value	I^2	*P* Value
T vs C	10	0.99 (0.88–1.10)	.80	Random	.01	59.21%	.39
CT vs CC	10	0.85 (0.70–1.02)	.09	Random	.00	65.98%	.52
TT vs CC	10	1.04 (0.84–1.28)	.72	Random	.04	48.18%	.48
Dominant (CT+TT vs CC)	10	0.89 (0.74–1.07)	.22	Random	.00	66.28%	.51
Overdominant (CT vs TT+CC)	10	0.84 (0.72–0.98)	.026	Random	.01	59.45%	.51
Recessive (TT vs CT+CC)	10	1.15 (1.01–1.30)	.032	Fixed	.15	31.77%	.59

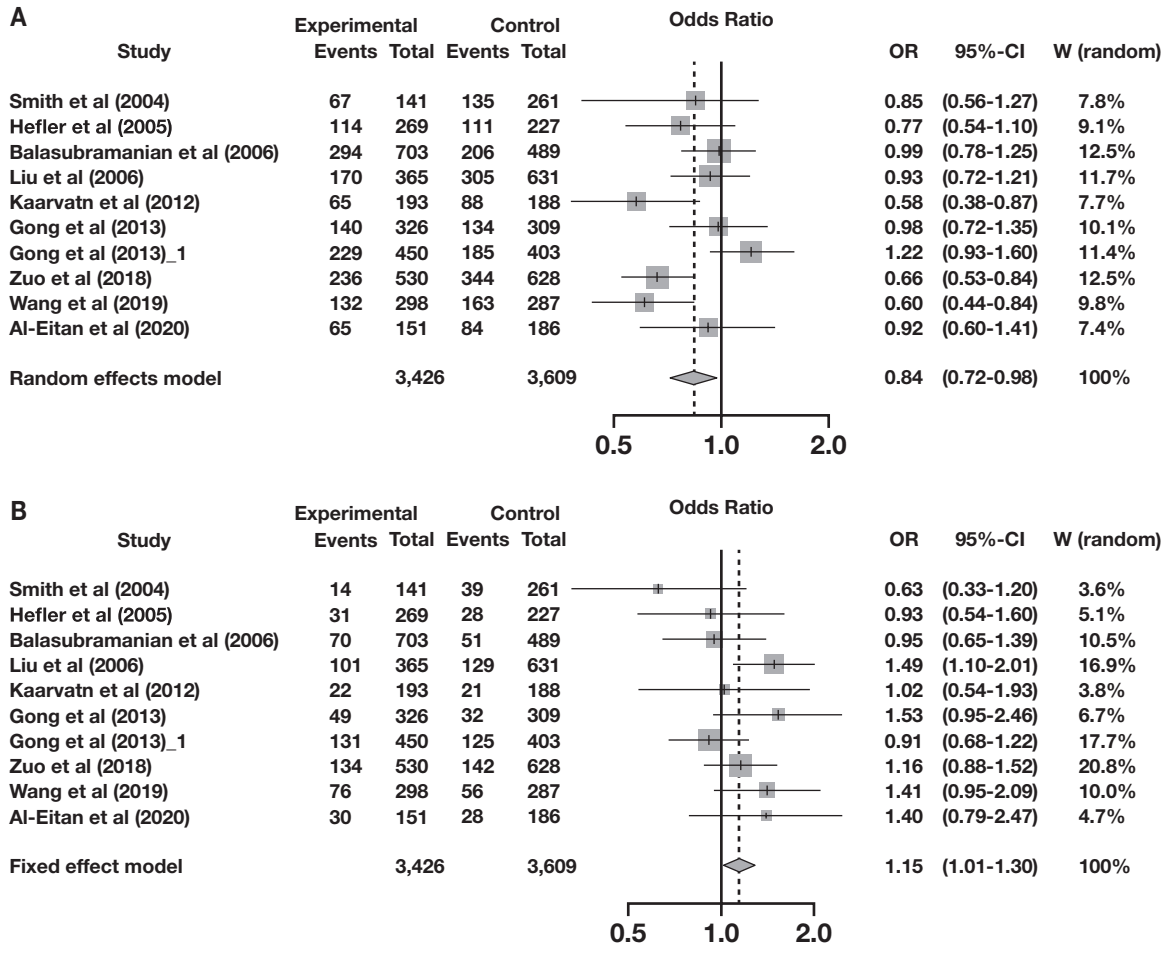


Figure 2

Forest plot describing pooled results for association between the *IL-1β* -511 C>T variant and BC risk under (a) overdominant and (b) recessive genetic models.

overdominant (OR, 0.84; 95% CI, 0.72–0.99; *P* =.04) models. In the Asian subgroup of patients, *IL-1β* -511C>T polymorphism conferred an enhanced risk of BC under the codominant CT vs TT (OR, 1.44; 95% CI, 1.20–1.73; *P* <.001) and recessive (OR, 1.33; 95% CI, 1.12–1.57; *P* =.001) genetic models. In addition, a protective role

against BC susceptibility was found in Asian patients regarding the overdominant model (OR, 0.76; 95% CI, 0.61–0.94; *P* =.011) (**Table 3**). With respect to genotyping methods, a significant association was observed between the *IL-1*β -511C>T variant and polymerase chain reaction-restriction fragment length polymorphism methods under the allelic, codominant homozygous, and recessive models (**Table 4**).

Analysis of Heterogeneity

The I^2 values for studies enrolled in the meta-analysis are shown in **Table 2**. The results indicated that heterogeneity existed among some studies (I^2 values > 50%). **Figure 3** shows the symmetrical shape of the funnel plots for the overdominant and recessive genetic models. Based on *P* values from the Egger funnel plot test and visual inspection, no publication bias was observed for the *IL-1*β -511C>T variant under the assessed genetic models. Assessment of quality for each study was carried out according to the QA criteria as described previously (Supplementary Table 1).[36] **Table 5** shows the stratified analysis of the *IL-1*β -511C>T polymorphism on BC risk according to QA scores. A significant association was found in high-quality studies between the variant and BC susceptibility under the recessive model (*P* =.01).

Sensitivity Analysis

The sensitivity analysis was conducted by sequential exclusion of a single study at a time to detect the potential effect of the individual study on the pooled ORs. Using this method, the pooled ORs remained consistently significant in the overdominant and recessive models (**Figure 4**), which suggests that the final pooled ORs were highly stable.

TSA and FPRP Analyses

In the present meta-analysis, 10 studies including 6940 individuals were included to assess the association of *IL-1*β -511C>T polymorphism with BC susceptibility. The cumulative *Z* curve did not cross the information size, and the total number of patients and control patients was less than the RIS (13,258). Therefore, the cumulative evidence was insufficient, and additional trials are necessary to validate the role of this genetic polymorphism in BC susceptibility (**Figure 5**). We also calculated the FPRP values for all detected significant findings. With the assumption of a prior probability of 0.25, the FPRP values were <0.2, implying that these significant associations were notable (**Table 6**).

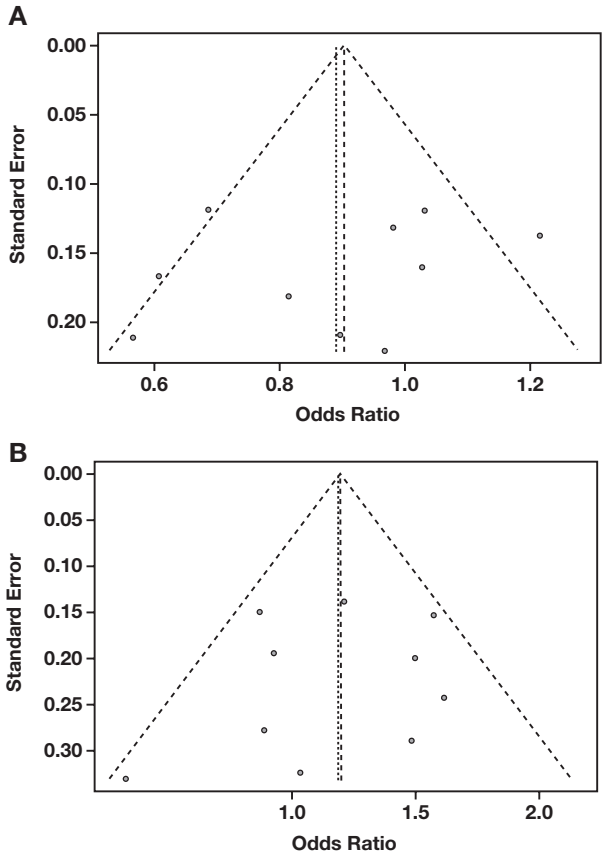


Figure 3

Funnel plot testing publication bias for association between the *IL-1*β -511 C>T variant and BC risk under (**A**) overdominant and (**B**) recessive contrasted models.

Discussion

In the present meta-analysis, 10 individual studies were included, and our pooled analysis indicated that the *IL-1*β -511C>T variant significantly increased BC risk under the recessive TT vs CT+CC model. Subgroup analysis showed the protective effect of this variant against BC risk in Caucasian patients but not in Asian patients.

DOI: 10.1093/labmed/lmaa055

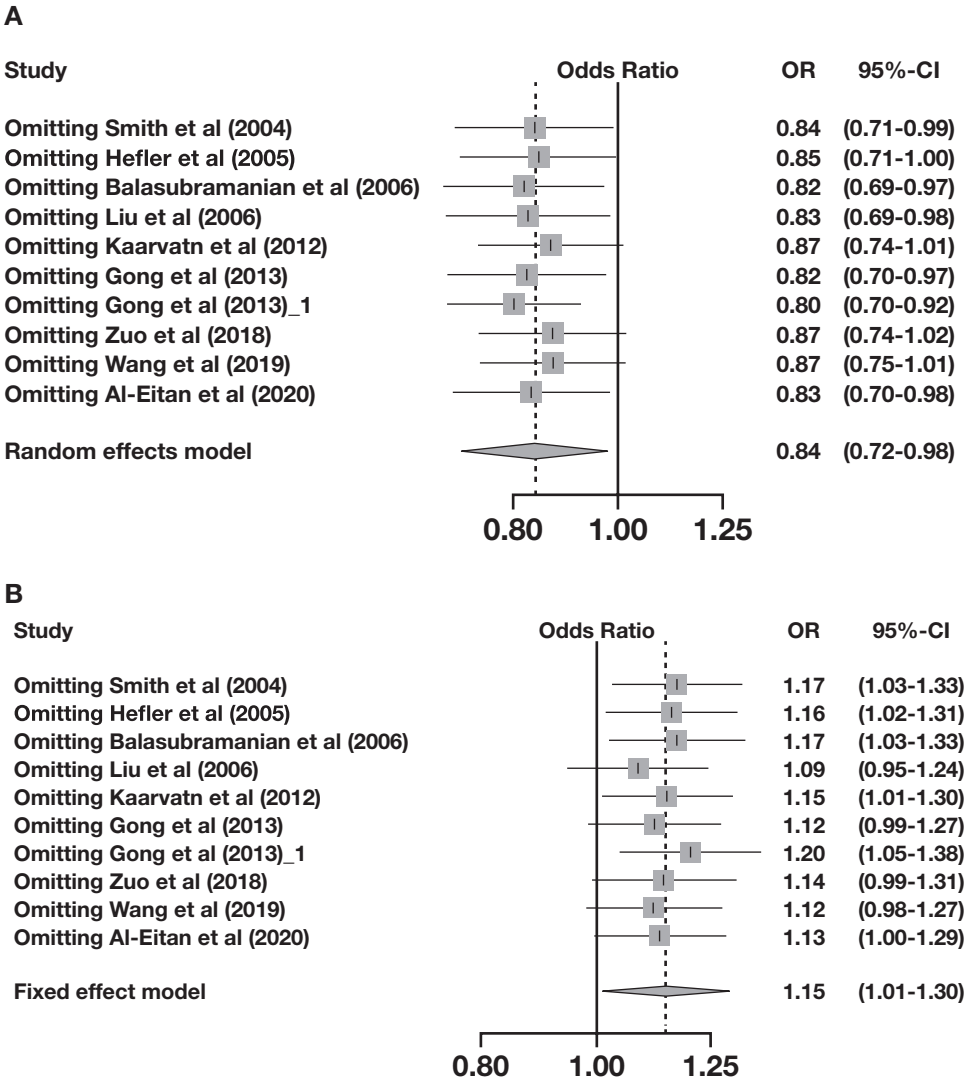


Figure 4

Sensitivity analysis through omitting each study to reflect influence of individual dataset to pooled ORs between the *IL-1*β -511 C>T variant and BC risk under (**A**) overdominant and (**B**) recessive models.

Critical roles of inflammation in tumor development and progression have been well established,[37] and genetic polymorphisms in the *IL-1* gene have been shown to affect the risk of developing BC in several case-control studies.[38-40] A previous meta-analysis by Xu et al[41] suggested that *IL-1*β -511C>T was not correlated with BC risk in the homozygous codominant TT vs CC, dominant TT+CT vs CC, codominant CT vs CC, and recessive TT vs CT+CC genetic models ($P > .05$). In another meta-analysis conducted by Liu, Wang, et al,[15] 4 studies were included, and the results suggested no significant association between the variant and BC susceptibility in the tested genetic models (codominant homozygous TT vs CC, codominant heterozygous CT vs CC, dominant TT+CT vs CC, and recessive TT vs CT+CC).[15]

The IL-1βlevels have been suggested to act as a predictive marker for developing bone metastasis in patients with BC.[16] High levels of IL-1R1, the type 1 receptor for IL-1β, has been associated with an enhanced risk

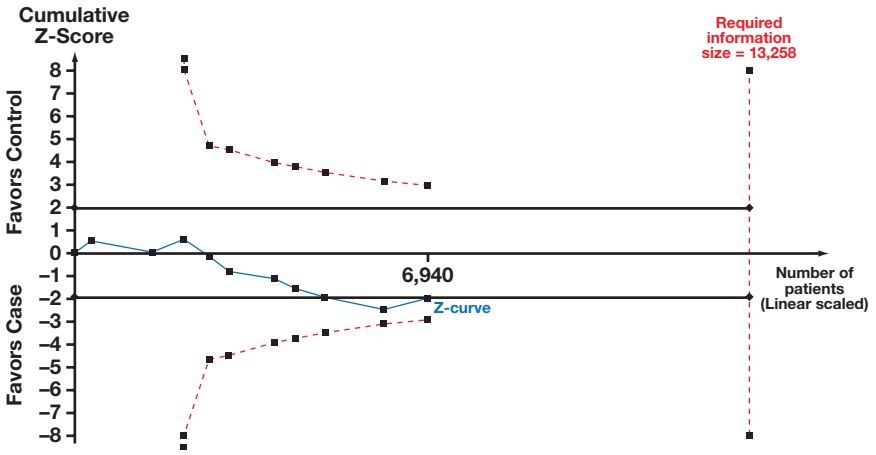


Figure 5

TSA of 10 studies reporting *IL-1*β -511 C>T polymorphism. The RIS was calculated using α = 0.05 (2-sided), β = 0.20 (power 80%), D2 = 57.0%, a relative risk increase of 10%, and an event proportion of 38.02% in the control arm. The blue cumulative Z-curve was constructed using a random-effects model.

Table 3. Stratified Analysis of the *IL-1*β -511 C>T Polymorphism on BC Risk in Different Ethnicities

Ethnicity	Number of Studies	Genetic Model	Association Test		Model	Heterogeneity Test		Egger Funnel Plot Test
			OR (95% CI)	*P* Value		*P* Value	I^2	*P* Value
Caucasian	4	T vs C	0.86 (0.76–0.97)	.02	Fixed	.29	19.41	.006
		CT vs CC	0.80 (0.68–0.95)	.01	Fixed	.12	48.98	.07
		TT vs CC	0.80 (0.61–1.05)	.11	Fixed	.55	0	.15
		Dominant (CT+TT vs CC)	0.80 (0.68–0.95)	.008	Fixed	.14	45.48	.03
		Overdominant (CT vs TT+CC)	0.84 (0.72–0.99)	.04	Fixed	.15	42.77	.16
		Recessive (TT vs CT+CC)	0.90 (0.69–1.16)	.40	Fixed	.70	0	.55
Asian	4	T vs C	1.07 (0.89–1.28)	.46	Random	.34	65.47	.73
		CT vs CC	0.81 (0.57–1.16)	.25	Random	.006	75.51	.59
		TT vs CC	1.17 (0.83–1.66)	.38	Random	.05	62.78	.72
		Dominant (CT+TT vs CC)	0.91 (0.64–1.29)	.61	Random	.08	77.63	.79
		Overdominant (CT vs TT+CC)	0.76 (0.61–0.94)	.011	Random	.09	52.39	.81
		Recessive (TT vs CT+CC)	1.33 (1.12–1.57)	.001	Fixed	.64	0	.59
American	2	T vs C	1.10 (0.95–1.27)	.21	Fixed	.25	23.02	N/A
		CT vs CC	1.18 (0.92–1.50)	.18	Fixed	.52	0	N/A
		TT vs CC	1.25 (0.92–1.69)	.15	Fixed	.23	31.73	N/A
		Dominant (CT+TT vs CC)	1.20 (0.95–1.50)	.13	Fixed	.97	0	N/A
		Overdominant (CT vs TT+CC)	1.11 (0.91–1.36)	.30	Fixed	.30	5.20	N/A
		Recessive (TT vs CT+CC)	1.14 (0.70–1.88)	.60	Random	.07	69.58	N/A

of developing primary tumors with poor prognosis, and decreased levels of IL-1β in cancer cells has been correlated with a good prognosis.[42] The gene *IL-1*β induces proinflammatory gene expression, including inducible nitric oxide synthase, cyclo-oxygenase type 2, and multiple cytokines that are crucial in the early phases of carcinogenesis.[42]

Because genetic polymorphisms in the *IL-1*β gene may affect the expression of *IL-1*β, it is important to assess the impact of *IL-1*β genetic variants on the risk of BC in a pooled analysis. In the current meta-analysis, we found a significant association between an *IL-1*β genetic variant and BC risk. However, some weaknesses of the study should be pointed out. First, although a significant relationship

DOI: 10.1093/labmed/lmaa055

Table 4. Stratified Analysis of the *IL-1*β -511 C>T Polymorphism on BC Risk in Different Genotyping Methods

Genotyping Method	Number of Studies	Genetic Model	Association Test		Model	Heterogeneity Test		Egger Funnel Plot Test
			OR (95% CI)	*P* Value		*P* Value	I^2	*P* Value
Mass array	4	T vs C	1.00 (0.91–1.11)	.93	Fixed	.24	29.32	.21
		CT vs CC	0.85 (0.58–1.24)	.40	Random	.001	80.70	.69
		TT vs CC	1.03 (0.85–1.26)	.74	Fixed	.21	33.81	.15
		Dominant (CT+TT vs CC)	0.91 (0.66–1.25)	.56	Random	.01	74.98	.64
		Overdominant (CT vs TT+CC)	0.83 (0.60–1.16)	.28	Random	0	81.49	.89
		Recessive (TT vs CT+CC)	1.15 (0.97–1.36)	.10	Fixed	.18	37.86	.25
PCR-RFLP	2	T vs C	1.25 (1.07–1.47)	.004	Fixed	.59	0	N/A
		CT vs CC	1.12 (0.87–1.45)	.38	Fixed	.65	0	N/A
		TT vs CC	1.58 (1.16–2.16)	.004	Fixed	.69	0	N/A
		Dominant (CT+TT vs CC)	1.25 (0.98–1.59)	.07	Fixed	.57	0	N/A
		Overdominant (CT vs TT+CC)	0.93 (0.74–1.16)	.51	Fixed	.95	0	N/A
		Recessive (TT vs CT+CC)	1.47 (1.13–1.91)	.004	Fixed	.85	0	N/A
TaqMan	2	T vs C	0.91 (0.78–1.06)	.22	Fixed	.14	53.82	N/A
		CT vs CC	0.75 (0.43–1.32)	.32	Random	.02	80.42	N/A
		TT vs CC	0.89 (0.64–1.26)	.52	Fixed	.64	0	N/A
		Dominant (CT+TT vs CC)	0.78 (0.49–1.26)	.31	random	.04	76.23	N/A
		Overdominant (CT vs TT+CC)	0.78 (0.46–1.31)	.34	Random	.03	79.63	N/A
		Recessive (TT vs CT+CC)	0.97 (0.70–1.34)	.85	Fixed	.84	0	N/A

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

Table 5. Stratified Analysis of the *IL-1*β -511 C>T Polymorphism on BC Risk According to QA Score

QA Score	Number of Studies	Genetic Model	Association Test		Model	Heterogeneity Test		Egger Funnel Plot Test
			OR (95% CI)	*P* Value		*P* Value	I^2	*P* Value
>9	8	T vs C	1.03 (0.92–1.15)	.60	Random	.03	55.39%	.94
		CT vs CC	0.88 (0.70–1.10)	.26	Random	.00	71.75%	.73
		TT vs CC	1.11 (0.96–1.29)	.17	Fixed	.10	41.26%	.80
		Dominant (CT+TT vs CC)	0.94 (0.77–1.15)	.56	Random	.00	69.56%	.84
		Overdominant (CT vs TT+CC)	0.84 (0.70–1.02)	.07	Random	.00	67.97%	.58
		Recessive (TT vs CT+CC)	1.19 (1.04–1.35)	.01	Fixed	.25	22.25%	.65
≤9	2	T vs C	0.79 (0.65–0.96)	.02	Fixed	.54	0.00%	N/A
		CT vs CC	0.72 (0.54–0.97)	.03	Fixed	.97	0.00%	N/A
		TT vs CC	0.66 (0.42–1.03)	.07	Fixed	.37	0.00%	N/A
		Dominant (CT+TT vs CC)	0.71 (0.54–0.94)	.01	Fixed	.74	0.00%	N/A
		Overdominant (CT vs TT+CC)	0.80 (0.61–1.05)	.10	Fixed	.73	0.00%	N/A
		Recessive (TT vs CT+CC)	0.79 (0.52–1.97)	.26	Fixed	.37	0.00%	N/A

was identified between the *IL-1*β genetic variant and BC risk, various possible causes of heterogeneity, including the demographic background of control patients, lifestyle, and environmental exposures, were not examined. Second, the study information included in this meta-analysis was based on unadjusted analyses, so we were unable to determine BC risk by considering environmental elements, age, family background, lifestyle, or additional risk factors that could have possible effects on the pooled results. Third, our meta-analysis only included the published literature, taking into account some pertinent relevant but unpublished studies. Finally, it is important to consider the possibility that the current pooled OR estimates were potentially skewed by the data from larger studies that were included in our meta-analysis.[33,34] Therefore, because of these inherent limitations, our meta-analysis should be interpreted with caution.

Table 6. False-Positive Report Probability Analysis of Noteworthy Results of *IL-1*β -511 C>T Polymorphism

Variable	OR (95% CI)	*P* Value	Power[a]	Prior Probability				
				0.25	0.1	0.01	0.001	0.0001
Overdominant (CT vs TT+CC)								
All	0.84 (0.72–0.98)	.0261		0.072	0.190	0.720	0.963	0.996
Caucasian patients	0.84 (0.72–0.99)	.04 1		0.107	0.265	0.798	0.976	0.998
Asian patients	0.76 (0.61–0.94)	.0111		0.032	0.090	0.521	0.917	0.991
Recessive (TT vs. CT+CC)								
All	1.14 (1.01–1.30)	.0310.502		0.156	0.357	0.859	0.984	0.998
Asian patients	1.33 (1.12–1.57)	.0010.997		0.003	0.009	0.090	0.501	0.909
PCR-RFLP	1.47 (1.13–1.91)	.0040.972		0.012	0.036	0.289	0.804	0.976
Score >9	1.19 (1.04–1.35)	.01 0.771		0.037	0.105	0.562	0.928	0.992

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.
[a]*Statistical power was calculated using the number of observations in the subgroup and the OR and* P *values in this table.*

Conclusion

Our study provides a comprehensive analysis of the link between the *IL-1*β -511C>T variant and the predisposition to BC. The results suggest that the *IL-1*β -511C>T variant significantly affects the risk of BC, particularly in Caucasian patient and Asian patient subgroups. More large and multicenter research is needed to confirm the prediagnostic influences of *IL-1*β gene polymorphism on the risk of BC.

Supplementary Data

Supplementary table can be found in the online version of this article at www.labmedicine.com. **LM**

References

1. Tao Z, Shi A, Lu C, Song T, Zhang Z, Zhao J. Breast cancer: epidemiology and etiology. *Cell Biochem Biophys.* 2015;72(2):333–338.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394–424.
3. Fenga C. Occupational exposure and risk of breast cancer. *Biomed Rep.* 2016;4(3):282–292.
4. Gracia-Aznarez FJ, Fernandez V, Pita G, et al. Whole exome sequencing suggests much of non-BRCA1/BRCA2 familial breast cancer is due to moderate and low penetrance susceptibility alleles. *PLoS One.* 2013;8(2):e55681.
5. Stevens KN, Vachon CM, Couch FJ. Genetic susceptibility to triple-negative breast cancer. *Cancer Res.* 2013;73(7):2025–2030.
6. Karimbux NY, Saraiya VM, Elangovan S, et al. Interleukin-1 gene polymorphisms and chronic periodontitis in adult whites: a systematic review and meta-analysis. *J Periodontol.* 2012;83(11):1407–1419.
7. Sims AM, Timms AE, Bruges-Armas J, et al. Prospective meta-analysis of interleukin 1 gene complex polymorphisms confirms associations with ankylosing spondylitis. *Ann Rheum Dis.* 2008;67(9):1305–1309.
8. Harrison P, Pointon JJ, Chapman K, Roddam A, Wordsworth BP. Interleukin-1 promoter region polymorphism role in rheumatoid arthritis: a meta-analysis of IL-1B-511A/G variant reveals association with rheumatoid arthritis. *Rheumatology (Oxford).* 2008;47(12):1768–1770.
9. Jotanovic Z, Mihelic R, Sestan B, Dembic Z. Role of interleukin-1 inhibitors in osteoarthritis: an evidence-based review. *Drugs Aging.* 2012;29(5):343–358.
10. Dai Z-J, Liu X-H, Ma Y-F, et al. Association between single nucleotide polymorphisms in DNA polymerase kappa gene and breast cancer risk in Chinese Han population: a STROBE-compliant observational study. *Medicine.* 2016;95(2):e2466.
11. Elaraj DM, Weinreich DM, Varghese S, et al. The role of interleukin 1 in growth and metastasis of human cancer xenografts. *Clin Cancer Res.* 2006;12(4):1088–1096.
12. Brough D, Tyrrell PJ, Allan SM. Regulation of interleukin-1 in acute brain injury. *Trends Pharmacol Sci.* 2011;32(10):617–622.
13. Ricote M, García-Tuñón I, Bethencourt FR, Fraile B, Paniagua R, Royuela M. Interleukin-1 (IL-1alpha and IL-1beta) and its receptors (IL-1RI, IL-1RII, and IL-1Ra) in prostate carcinoma. *Cancer.* 2004;100(7):1388–1396.
14. Xia P, Jin T, Geng T, et al. Polymorphisms in ESR1 and FLJ43663 are associated with breast cancer risk in the Han population. *Tumour Biol.* 2014;35(3):2187–2190.
15. Liu X, Wang Z, Yu J, Lei G, Wang S. Three polymorphisms in interleukin-1β gene and risk for breast cancer: a meta-analysis. *Breast Cancer Res Treat.* 2010;124(3):821–825.
16. Holen I, Lefley DV, Francis SE, et al. IL-1 drives breast cancer growth and bone metastasis in vivo. *Oncotarget.* 2016;7(46):75571–75584.
17. Xia P, Li B, Geng T, et al. FGFR2 gene polymorphisms are associated with breast cancer risk in the Han Chinese population. *Am J Cancer Res.* 2015;5(5):1854–1861.
18. Lacruz-Guzmán D, Torres-Moreno D, Pedrero F, et al. Influence of polymorphisms and TNF and IL1β serum concentration on the infliximab response in Crohn's disease and ulcerative colitis. *Eur J Clin Pharmacol.* 2013;69(3):431–438.
19. 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature.* 2015;526(7571):68–74.
20. Arend WP. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev.* 2002;13(4–5):323–340.
21. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution

DOI: 10.1093/labmed/lmaa055

of common variants to susceptibility to common disease. *Nat Genet.* 2003;33(2):177–182.

22. He J, Liao X-Y, Zhu J-H, et al. Association of MTHFR C677T and A1298C polymorphisms with non-Hodgkin lymphoma susceptibility: evidence from a meta-analysis. *Sci Rep.* 2014;4(1):1–9.
23. Martorell-Marugan J, Toro-Dominguez D, Alarcon-Riquelme ME, Carmona-Saez P. MetaGenyo: a web tool for meta-analysis of genetic association studies. *BMC Bioinformatics.* 2017;18(1):563.
24. Yang F, Wei K, Qin Z, Shao C, Shu Y, Shen H. Association between TNF-α-308G/A polymorphism and esophageal cancer risk: an updated meta-analysis and trial sequential analysis. *J Cancer.* 2019;10(5):1086–1096.
25. Brok J, Thorlund K, Gluud C, Wetterslev J. Trial sequential analysis reveals insufficient information size and potentially false positive results in many meta-analyses. *J Clin Epidemiol.* 2008;61(8):763–769.
26. Fu W, Zhuo ZJ, Chen YC, et al. NFKB1 -94insertion/deletion ATTG polymorphism and cancer risk: evidence from 50 case-control studies. *Oncotarget.* 2017;8(6):9806–9822.
27. Smith KC, Bateman AC, Fussell HM, Howell WM. Cytokine gene polymorphisms and breast cancer susceptibility and prognosis. *Eur J Immunogenet.* 2004;31(4):167–173.
28. Hefler LA, Grimm C, Lantzsch T, et al. Interleukin-1 and interleukin-6 gene polymorphisms and the risk of breast cancer in Caucasian women. *Clin Cancer Res.* 2005;11(16):5718–5721.
29. Balasubramanian SP, Azmy IA, Higham SE, et al. Interleukin gene polymorphisms and breast cancer: a case control study and systematic literature review. *BMC Cancer.* 2006;6(1):188.
30. Liu J, Zhai X, Jin G, et al. Functional variants in the promoter of interleukin-1beta are associated with an increased risk of breast cancer: a case-control analysis in a Chinese population. *Int J Cancer.* 2006;118(10):2554–2558.
31. Kaarvatn MH, Krog Eftedal R, Vrbanec J, et al. Interleukin-1 gene locus polymorphisms are associated with risk to breast cancer in Croatian population. *Period Biol*. 2012;114(4):497–503.
32. Gong Z, Quan L, Yao S, et al. Innate immunity pathways and breast cancer risk in African American and European-American women in the Women's Circle of Health Study (WCHS). *PLoS One.* 2013;8(8):e72619.
33. Zuo X, Li M, Yang Y, et al. Interleukin gene polymorphisms in Chinese Han population with breast cancer, a case-control study. *Oncotarget.* 2018;9(26):17994–18001.
34. Wang J, Shi Y, Wang G, Dong S, Yang D, Zuo X. The association between interleukin-1 polymorphisms and their protein expression in Chinese Han patients with breast cancer. *Mol Genet Genomic Med.* 2019;7(8):e804.
35. Al-Eitan LN, Al-Ahmad BH, Almomani FA. The association of IL-1 and HRAS gene polymorphisms with breast cancer susceptibility in a Jordanian population of Arab descent: a genotype-phenotype study. *Cancers.* 2020;12(2):283.
36. Cai J, Ye Q, Luo S, et al. CASP8 -652 6N insertion/deletion polymorphism and overall cancer risk: evidence from 49 studies. *Oncotarget.* 2017;8(34):56780–56790.
37. Tower H, Ruppert M, Britt K. The immune microenvironment of breast cancer progression. *Cancers.* 2019;11(9):1375.
38. Snoussi K, Strosberg AD, Bouaouina N, Ben Ahmed S, Chouchane L. Genetic variation in pro-inflammatory cytokines (interleukin-1beta, interleukin-1alpha and interleukin-6) associated with the aggressive forms, survival, and relapse prediction of breast carcinoma. *Eur Cytokine Netw.* 2005;16(4):253–260.
39. Lee KM, Park SK, Hamajima N, et al. Genetic polymorphisms of interleukin-1 beta (IL-1B) and IL-1 receptor antagonist (IL-1RN) and breast cancer risk in Korean women. *Breast Cancer Res Treat.* 2006;96(2):197–202.
40. Grimm C, Kantelhardt E, Heinze G, et al. The prognostic value of four interleukin-1 gene polymorphisms in Caucasian women with breast cancer: a multicenter study. *BMC Cancer.* 2009;9(1):78.
41. Xu J, Yin Z, Cao S, et al. Systematic review and meta-analysis on the association between IL-1B polymorphisms and cancer risk. *PLoS One.* 2013;8(5):e63654.
42. Yencilek F, Yildirim A, Yilmaz SG, et al. Investigation of interleukin-1β polymorphisms in prostate cancer. *Anticancer Res.* 2015;35(11):6057–6061.

Science

Urine S-Adenosylmethionine are Related to Degree of Renal Insufficiency in Patients with Chronic Kidney Disease

Maria Petrovna Kruglova, MD,[1] *Alexander Vladimirovich Ivanov, PhD,*[2*] *Edward Danielevich Virus, PhD,*[2] *Polina Olegovna Bulgakova, MD,*[2] *Andrey Segeevich Samokhin, PhD,*[2] *Anatolij Nikolaevich Fedoseev, ScD,*[3] *Sergej Vital'evich Grachev, ScD,*[1] *Aslan Amirkhanovich Kubatiev, ScD*[2,4]

Laboratory Medicine 2021;52:47-56

DOI: 10.1093/labmed/lmaa034

ABSTRACT

Objective: To determine whether urine S-adenosylmethionine (SAM) might be an indicator of chronic kidney disease (CKD).

Methods: We investigated urine levels of SAM and related metabolites (S-adenosylhomocysteine and homocysteine cysteine) in 62 patients (average age, 65.9 years) with CKD (stages II–V).

Results: Patients with stages III–V CKD stages have significantly decreased urine levels and SAM/S-adenosylhomocysteine ratio and also cysteine/homocysteine ratio in blood plasma (P <.05), compared with patients with stage II CKD. Urine SAM levels allowed us to distinguish patients with mildly decreased kidney function from those with moderate to severe renal impairment (AUC, 0.791; sensitivity, 85%; specificity, 78.6%).

Conclusions: Our study results demonstrate that urine SAM is a potent biomarker for monitoring renal function decline at early CKD stages. Urine SAM testing confers an additional advantage to healthcare professionals in that it is noninvasive.

Keywords: chronic kidney disease, cysteine, homocysteine, S-adenosylhomocysteine, S-adenosylmethionine, urine

Chronic kidney disease (CKD) is a disease characterized by a progressive loss of kidney function during several months or years. In assessing renal function, a leading role is played by such indicators as estimated glomerular filtration rate (eGFR). CKD is characterized by eGFR of less than 60 mL divided by minutes divided by 1.73 m^2 and signs of kidney injury with a duration of at least 3 months.[1] The calculation of eGFR by examining the level of plasma creatinine (Crn) metabolite not reabsorbed by the kidneys, taking into account additional factors (age, sex, and race), was the most widespread in clinical practice.[2,3] There are also other, more complicated methods for eGFR determination using endogenous or exogenous markers (cystatin C, kidney injury molecule–1 [KIM-1], uric acid, symmetric dimethiarginine, etc). However, these indicators are not prognostic markers of CKD, and assaying for them requires invasive intervention.[2,4–7]

The simplicity of obtaining biomaterial is the main advantage of noninvasive markers for monitoring the progression of CKD in a wide range of patients. Therefore, scientists have devoted much attention to searching for them.

In addition to eGFR, it was previously proposed[8] to use the urine albumin to Crn ratio for a more accurate classification

Abbreviations:

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; Crn, creatinine; KIM-1, kidney injury molecule–1; NGAL, neutrophil gelatinase-associated lipocalin; EGF, epidermal growth factor; CADM1, cell adhesion molecule 1; CE, capillary electrophoresis; MS, mass spectrometry; LC, liquid chromatography; mRNA, messenger RNA; FRAP, ferric ion reducing antioxidant parameter; Hcy, homocysteine; HHcy, hyperhomocysteinemia; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; HIV, human immunodeficiency virus; Cys, cysteine; CE, capillary electrophoresis; SPE, solid-phase extraction; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; … , nonapplicable

[1]I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia, [2]Department of Molecular and Cell Pathophysiology, Institute of General Pathology and Pathophysiology, Moscow, Russia, [3]City Clinical Hospital No. 24 of Moscow Healthcare Department, Moscow, Russia, [4]Russian Medical Academy of Postdoctoral Education, Moscow, Russia

*To whom correspondence should be addressed.
Ivanov_av82@mail.ru

of CKD. Separately, this indicator was also proposed to be used for diagnosis in the early stages of diabetic kidney disease. However, this indicator was characterized by low sensitivity because albuminuria was not observed in a statistically significant number of patients.[8] Later, a negative association between protein levels in the urine (neutrophil gelatinase-associated lipocalin [NGAL] and retinol-binding protein) and eGFR was found.[9]

An increase in the level of epidermal growth factor (EGF) in urine was shown[10] to be associated with a rapid drop in eGFR. A negative association of cell adhesion molecule 1 (CADM1) in urine with eGFR was shown,[11] which led the authors to consider this compound as an indicator of tubulointerstitial damage to the kidneys. At the same time, this association was observed only at an increased (>1.6 mkg/L) level of CADM1 and was not specific to changes in GFR at its normal level.

Thanks to the development of proteomic methods, it became possible to comprehensively study the protein and peptide composition of urine for diagnostic purposes. The study of urine peptides using capillary electrophoresis (CE), coupled with the mass-spectrometry (MS) method, made it possible to diagnose CKD with a high degree of reliability, as well as diagnosing the progression of CKD and differentiating its various subtypes.[12–14] Based on this method, Pontillo et al proposed[15] to use peptides—collagen fragments in the urine—as early CKD markers that showed high sensitivity in individuals with eGFR of greater than 60 mL per minute per 1.73 m^2. These entities were also prominent biomarkers for 3 to 5 years before onset of macroalbuminuria.[16] Liquid chromatography (LC), coupled with the MS method, was used to profile urine glycoproteins.[17] Multiple proteins unique to CKD that may be of interest as biomarkers were found. However, these approaches are costly and time-consuming, so they are not yet available for clinics conducting large-scale research.

In recent years, various types of RNA in urine have been considered as promising markers, specifically messenger RNA (mRNA) of hepatocyte growth factor and connective tissue growth factor CNN2.[18] Alterations of several urine metabolites (acotinic, glycolic, citric, homovanilic acids, 2-ethyl-3-OH-propionate) were associated with early structural changes in the glomerular apparatus in individuals with type 2 diabetes.[19] It was proposed[20] to use the total antioxidant potential determined by the FRAP (ferric ion reducing antioxidant parameter) method in stimulated saliva as a noninvasive indicator of CKD and its progression in children. It is reported that this method allowed patients with mild to moderate kidney function to be differentiated from those with severe renal impairment with 100% specificity and sensitivity. Thus, the search for new noninvasive, pathogenetically based markers for large-scale studies of patients with CKD remains relevant.

In the pathogenesis of CKD, a major role is played by disruption of the methylation processes of various substrates, particularly DNA.[21] An association of DNA methylation with a rapid decrease in renal function has been shown.[22] Also, it has recently been shown[23] that the DNA methylation marker is 5-methyl-2′-deoxycitidine was significantly associated with the prediction of later (IV–V) CKD stages, compared with stages I through III. This metabolite was proposed as a potent biomarker for predicting the renal outcome in patients with CKD.

SAM is a universal carrier of methyl groups; when giving up a methyl group, SAM turns into SAH (a competitive inhibitor of methyltransferases), which then turns into homocysteine (Hcy). An increased level of the lattermost entity (hyperhomocysteinemia [HHcy]) is observed in a significant number of patients with CKD:more than 80% at stages IV through V.[24] Two studies[25,26] have identified the prognostic potential of HHcy in relation to the risk of developing and progression of CKD, and in relation to the risk of developing cardiovascular complications in CKD in the absence of special Hcy-reducing interventions. However, it is not yet clear whether HHcy is the cause or a marker of CKD and concomitant vascular diseases, and it is unclear whether the increase in plasma Hcy is due to decreased renal filtration/perfusion or due to a systemic violation of the Hcy-utilization pathway.[27]

Like other amino acids, Hcy is almost completely reabsorbed from primary urine (≥99%) in normal conditions.[27] Patients with severe CKD showed a decrease in Hcy clearance by approximately 30% and the completeness of its reabsorption to 85%. However, previously, there were no differences in the urine Hcy level between healthy volunteers and patients with CKD.[28,29] However, the precursors of Hcy, namely, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), have a high clearance, 93% and 39%, respectively, relative to Crn, which justifies study of the levels of these analytes in urine as indicators of CKD and information that informs prognosis of its course.[30]

Although many of us[29] previously showed that urinary SAH levels in patients with CKD did not differ from those levels in control individuals, a significant decrease in SAM levels and SAM/SAH ratios in urine was revealed. In this regard, it is of interest to use these indicators for noninvasive prediction of the course of CKD and assessment of HHcy levels. However, to our knowledge, it has not yet been investigated whether there is a relationship between SAM in the urine and the progression of CKD. Therefore, the aim of this work was to study the association between urine SAM and SAH levels, plasma homocysteine, and the state of renal function in patients with CKD at its various stages.

Materials and Methods

Patients

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, as well as with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This Study Protocol was approved by the Clinical Hospital No. 24 of Moscow Healthcare Department ethics committee.

In this randomized case-control study, we included 62 patients with chronic tubulointerstitial nephritis (CTIN; n = 50) and chronic glomerulonephritis (n = 12) enrolled in planned treatment, with an average age of 65.9 years (37–81 years); 43% of those patients are male.

A total of 20 patients had stage II CKD, 17 patients had stage III CKD, 14 patients had stage IV CKD, and 11 patients had stage V CKD. Diabetes mellitus was diagnosed in 29% of patients. Exclusion criteria included pregnancy, lactation, HIV (human immunodeficiency virus) infection, severe somatic pathologic manifestations (oncological diseases, severe chronic respiratory failure, and/or liver failure), severe purulent-septic diseases, cancer, and stroke or myocardial infarction within the previous 4 months. CKD was classified according to the National Kidney Foundation into stages based on GFR plus pathologic abnormalities or markers of kidney damage.[31] GFR was estimated according to the CKD-EPI (exocrine pancreatic insufficiency) formula.[32]

Laboratory Procedures

Urine and venous blood specimens were collected in the morning from fasting volunteers. Blood specimens with 0.38% sodium citrate levels were immediately centrifuged for 10 minutes at 2000 *g*, and the obtained plasma was chilled on ice. One mL of 100 mM hydrochloric acid was immediately added to 10 mL of the urine specimen. Plasma and urine aliquots were frozen within 1 hour and stored at –75°C for further analysis.

Plasma and urine cysteine (Cys) and Hcy were determined using capillary electrophoresis (CE) methods[33,34]; precision was within 3%. SAM and SAH levels in urine were determined, with precision of 9.1% and 7.6%, respectively.[35] Crn levels in urine were determined with precision of 3.8%.[36] The CE 3D system (Agilent Technologies) was used with silica capillary tubing of 30.5 cm total length and 50 μm internal diameter.

For rapid Cys and Hcy determination in plasma, 100 μL of plasma was mixed with 20 μL dithiothreitol (25 mM) and 10 μL of penicillamine (internal standard, 0.45 mM) were added. The mixture was incubated at 37°C for 15 minutes. We added 450 μL of 1,1′-thiocarbonyldiimidazole (35 mM) in acetonitrile, incubated the specimen at 4°C for 30 minutes, and centrifuged it for 5 minutes at 15,000 *g* before analysis. The specimen was injected at –13 kV for 30 seconds and then potassium hydroxide (0.1 M) was injected for 60 seconds at the same voltage. CE was performed using triethanolamine (0.1 M) with formic acid (0.15 M) and hexadecyltrimethylammonium bromide (50 μM) as the background electrolyte. The absorption wavelength was 285 nm (reference value, 330 nm).

For Cys and Hcy determination in urine, specimens were treated in the same way and then 20 μL of hydrochloric acid (1 M) and 0.1 mL of choloform were added to a 0.2 mL specimen for liquid-to-liquid extraction. The upper (water-rich) phase of the mixture was then discarded, and the lower phase (acetonitrile + chloroform) was evaporated and suspended in 50 μL of water. Specimens were centrifuged at 3000 *g* for 1 minute before analysis. The specimen was injected at 50 mbar for 45 seconds. Then, potassium hydroxide (0.5 M) was injected for 30 seconds at –17 kV. CE was performed at the same voltage. The background electrolyte was phosphoric acid (0.1 M) with triethanolamine (0.03 M), hexadecyltrimethylammonium bromide (25 μM), sodium dodecyl sulfonate (2.5 μM), and

DOI: 10.1093/labmed/lmaa034

polyethylene glycol 600 (2.5% volume/volume), with pH of 2 for the entire solution.

For SAM and SAH determination, 0.2 mL of disodium phosphate (0.2 M) was added to 2.2 mL of the urine specimen. This mixture was passed through a solid-phase extraction (SPE) cartridge with Bond Elute PBA 100 mg phase (Agilent Technologies) for analyte extraction. The phase was flushed with 0.01 M disodium phosphate, 0.01 M ammonium acetate (2 mL). SAM and SAH were desorbed with 1 mL of hydrochloric acid (0.1 M). 10 μL sodium chloride (1 M) with meglumine (0.1 M), and 5 μL sodium hydroxide (0.5 M) were mixed with 80 μL of specimen material before analysis.

The specimen was injected at 50 mbar during a period of 60 seconds. The CE voltage was 15 kV, the background electrolyte was formic acid (1 M) with Tris (20 mM), pH 2.2. The absorption wavelength was 254 nm; the reference wavelength was 290 nm.

For determination of Crn level in urine, specimens were diluted 20-fold with imidazole (0.4 M). The specimen was injected at 50 mbar for 5 seconds at the anodic end of the capillary. CE was performed at –15 kV using sodium dihydrogen phosphate (0.2 M) with acetic acid (0.2 M) as background electrolyte. The absorption wavelength was 200 nm.

Statistical Analysis

Primary processing of electropherograms was carried out by using Data Analysis ChemStation software, version B.01.03 (Agilent Technologies). Data were collected and analyzed using SPSS software, version 22 (IBM Corporation). Because all data were not normally distributed, it was expressed as median (1st and 3rd quartile). Differences between groups were tested for significance using nonparametric Mann-Whitney U and Kruskal-Wallis testing. Correlations were performed by using Spearman (ρ) rank testing. The observed difference was considered to be significant at $P < .05$. In case of multiple comparisons, we used the Holm-Bonferroni method for the correction of P values. The receiver operating characteristic (ROC) curve was used to estimate predictors of CKD stage among the study population.

Results

The main characteristics of the study population are listed in **Table 1**, according to the stage of renal dysfunction. As we observed, a stepwise decrease in eGFR and an increase in Crn level in plasma are accompanied by an increase in urea level, as well as a noticeable decrease in hemoglobin content in the blood.

The levels of metabolites of interest in plasma and urine are presented in **Table 2**. Most of the patients had mild-to-moderate HHcy (Hcy, 15–30 μM). HHcy was observed in 45%, 82%, 86%, and 100 % of patients with CKD stage II, III, IV and V , respectively. We note here that a significant

Table 1. Baseline Characteristics of CKD and Control Groups

Variable	CKD Stage				*P* Value[a]
	II	III	IV	V	
No.	20	17	14	11	…
Age, y, median (1st–3rd quartile)	64.5 (62–67)	68 (64–70)	65.5 (60–75)	69 (66–76)	.30
Sex, % male	50	47	43	27	…
Diabetes mellitus, %	25	23.5	29	45.5	…
eGFR, mL/min/1.73 m^2	65 (60–70)	41.9 (38.6–48.9)	22.4 (18.6–27.7)	8.2 (7.6–9.8)	<.001
Plasma albumin, g/L	42.2 (37.5–45.6)	42.4 (39.2–45.8)	42.7 (41.0–44.2)	37.8 (35.2–42.9)	.38
Plasma Crn, μM	94 (82.5–101._]	122 (112–150)	219 (175–259)	474 (376–580)	<.001
Plasma urea, mM	5.8 (4.2–6.4)	8 (6.8–11.9)	13 (9.4–14.7)	21.5 (18.4–27.2)	<.001
Urine Crn, mM	3.5 (1.8–7.1)	1.8 (1.2–3.3)	3.4 (1.3–5.4)	1.7 (0.9–2.2)	.11
Plasma cholesterol, mM	5.7 (4.9–7.2)	5.1 (4.5–5.9)	5.4 (4.4–5.7)	4.3 (3.8–6.0)	.15
Blood glucose, mM	5.7 (5.2–6.4)	5.5 (5.1–6.0)	5.5 (5.4–6.0)	5.6 (5.1–6.1)	.85
Blood hemoglobin, g/L	144 (139–149)	134 (123–145)	116 (100–124)	100.5 (93.0–111.5)	<.001

CKD, chronic kidney disease; … , nonapplicable; eGFR, estimated glomerular filtration rate; Crn, creatinine.
[a]*Via corrected Kruskal-Wallis testing.*

Table 2. Levels of Homocysteine Metabolites in Plasma and Urine[a]

Matrix	Variable, μM	CKD Stage (Median [1st–3rd Quartiles])				*P* Value[b]
		II	III	IV	V	
Plasma	Hcy	14.0 (11.3–24.0)	18.8 (16.6–23.2)	25.4 (16.8 –36.4)	30.1 (23.3–32.3)[c]	.08
	Cys	359 (313–445)	347 (297–399)	401 (337–489]	384 (320–465)	.96
	Cys/Hcy	*26.0 (19.8–30.5)*	*18.3 (14.2–22.3)[c]*	*17.3 (13.6–22.1)[c]*	*11.4 (9.9–15.9)[c]*	*.006*
Urine	Hcy	1.82 (0.89–2.83)	1.55 (0.81–2.31)	1.19 (0.80–1.67)	1.32 (0.99–4.72)	.89
	Cys	96.7 (51.8–162.5)	27.0 (22.5–59.5)[c]	62.9 (31.7–114.6)	79.6 (50.9–269)	.18
	SAM	*7.2 (4.6–10.6)*	*3.1 (2.0–4.3)[c]*	*2.8 (1.6–4.4)[c]*	*1.8 (1.5–2.7)[c]*	*.007*
	SAH	0.97 (0.65–1.33)	1.06 (0.65–1.45)	1.35 (0.57–3.08)	0.66 (0.30–1.60)	>.99
	SAM/SAH	6.84 (4.53–9.00)	3.00 (1.84–4.89)[c]	2.15 (1.37–3.66)[c]	2.51 (1.42–4.74)[c]	.06

CKD, chronic kidney disease; Cys, cysteine; Hcy, homocysteine; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.
[a]*Italics indicate significant difference.*
[b]*Kruscal-corrected.*
[c]*P <.05 (via Mann-Whitney testing), compared with CKD stage II.*

difference in Hcy plasma level was observed only between the patients with CKD disease at stages II and V ($P = .03$; **Figure 1A**). We did not observe differences among groups of patients in plasma Cys levels; however, the Cys/Hcy ratio significantly decreased in patients with CKD stages III through V, compared with those having stage II CKD ($P \leq .02$; **Figure 1B**).

There was no change in urine Cys and Hcy levels in patients with CKD stages II through V, except that in patients with stage III disease, a significant decrease in the level of Cys was observed ($P = .02$; **Table 2**). There were also no significant differences between urine SAH levels among groups with CKD at different stages. In contrast, there was a significant decrease in urine SAM levels and the SAM/SAH ratio in patients with CKD at stages III through V, compared with those whose CKD is at stage II (**Figures 1C** and **1D**). In this case, differences in these indicators between stages III and V were not revealed.

Associations between the analyzed parameters in all patients with CKD are presented in **Table 3**. eGFR had a pronounced correlation with plasma Hcy and urine SAM levels. However, we observed no reliable association between these metabolites. Also, urinary SAM with Crn and Cys with Hcy levels in urine and plasma were associated. However, we found no relationship between plasma and urinary levels of these aminothiols. Also, we discovered no reliable association of urine SAH level with other analyzed metabolites.

We detected positive correlations of SAM with Crn levels in urine, Cys with Hcy levels in urine, and Cys with Hcy in plasma in the group of patients with CKD at stage II. However, no association of eGFR with any of these indicators was found (**Table 3**).

In patients with CKD at stages III through V, a reliable association of eGFR only with plasma Hcy level was revealed (**Table 3**). An association between SAM and Crn levels in urine, unlike in patients with stage II CKD, was not observed. However, there was also a stable correlation between the levels of Cys and Hcy in urine and plasma separately.

We performed a ROC analysis of urine SAM, SAM/SAH ratio, and plasma Cys/Hcy ratio to assess the effectiveness of these potential markers in distinguishing mild (stage II) from moderate and severe (stages III–V) CKD (**Figure 2**). These analytes yield an area under the curve (AUC) of 0.78 to 0.80 (95% confidence interval [CI], 0.65–0.95). The SAM cutoff level in the urine was 4.26 μM, and the sensitivity and specificity values were 0.85 and 0.786, respectively. The SAM/SAH cutoff level was 4.19 (sensitivity and specificity, 0.84 and 0.75, respectively). The Cys/Hcy cutoff value in plasma was 17.83 (sensitivity and specificity, 0.79 and 0.69, respectively). The AUC of Hcy in plasma was 0.692 (95% CI, 0.528–0.855; $P = .02$), and its cutoff value was 18.71 μM (sensitivity and specificity, 0.63 and 0.67, respectively).

Discussion

Independent (from classical risk factors such as age, hypertension, obesity, and diabetes) association of plasma Hcy

Lab Medicine 2021;52;47–56 51
DOI: 10.1093/labmed/lmaa034

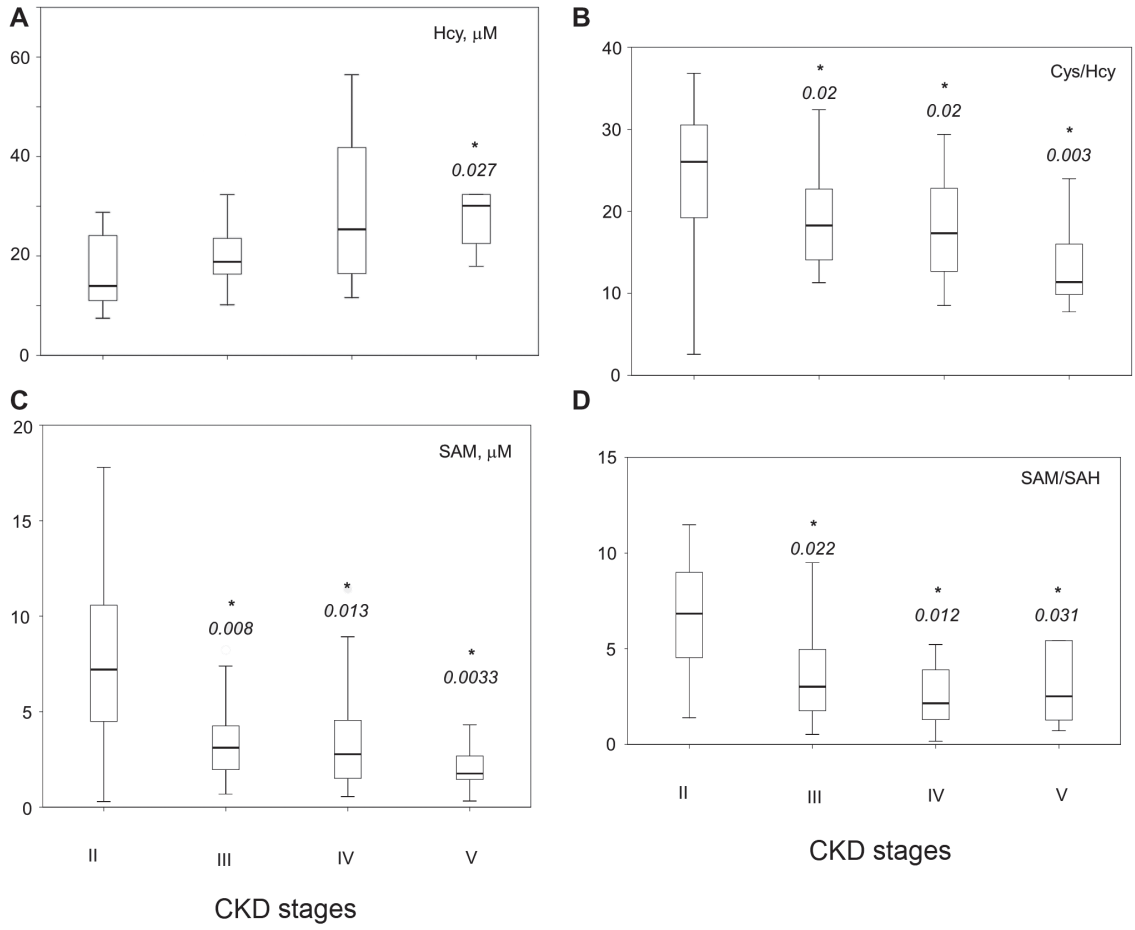


Figure 1

Plasma and urine metabolites at different stages of chronic kidney disease (CKD). **A**, Plasma homocysteine (Hcy). **B**, Plasma cysteine (Cys)/Hcy. **C**, Urine S-adenosylmethionine (SAM). **D**, Urine SAM/S-adenosylhomocysteine (SAH). * - compared to II stage.

levels with CKD was shown in many study reports.[37,38] Accordingly, it was previously demonstrated[25] that an increase in plasma Hcy level is associated with accelerated decline in renal function. Plasma Cys and Hcy levels are positively correlated with CKD stages in patients with diabetes mellitus.[39,40] However, the prognostic potential of Hcy in relation to CKD is still a matter of debate. This situation can be partially explained by the fact that many factors influence the level of Hcy; these factors vary widely in the population. We found a credible difference in plasma Hcy level only between CKD stages II and V, and did not find any differences in plasma Cys level among all groups of patients with CKD, although negative correlations between Cys and Hcy in plasma with eGFR were present in patients with CKD at stages III through V.

Despite this information, the plasma Cys/Hcy ratio turned out to be a more sensitive indicator because it had already significantly decreased at stage III of CKD. Still, we did not find any significant decrease in the level of Cys in the blood plasma in patients, although we observed a negative association of this indicator with eGFR in patients whose CKD was at stages III through V. In this regard, we note that a close negative association between the plasma Cys/cystathionine ratio and the stages of CKD had been shown previously in the literature.[39] Cystathionine is an intermediate in the conversion of Hcy to Cys; it is formed under the action of cystathionine β-synthase in the first stage of transsulfuration and is normally considered to be a speed-limiting step in this pathway. This information may indicate that at stage III of CKD, systemic inhibition of transulfuration

Table 3. Spearman Correlation Coefficients in Patients

Analyte	eGFR	Urine Crn	Urine SAM	Urine SAH	Urine Cys	Urine Hcy	Plasma Cys	Plasma Hcy
				CKD Stages II–V				
eGFR	…	0.183	0.513[a]	–0.04598	–0.0102	–0.00181	–0.197	–0.480[a]
Urine Crn		…	0.590[b]	0.102	0.326	0.35895	–0.0613	–0.203
Urine SAM			…	0.281	0.224	0.09259	–0.131	–0.305
Urine SAH				…	–0.004	0.0113	–0.0408	0.0835
Urine Cys					…	0.647[b]	0.0325	0.003
Urine Hcy						…	0.074	0.099
Plasma Cys							…	0.617[n]
CKD Stage II								
eGFR	…-	–0.002	–0.017	–0.311	0.114	0.130	–0.030	0.086
Urine Crn		…	0.824[b]	0.353	0.522[a]	0.186	–0.296	–0.487[a]
Urine SAM			…	0.665[a]	0.409	0.215	–0.099	–0.296
Urine SAH				…	0.209	0.162	0.158	0.135
Urine Cys					…	0.719[a]	0.071	–0.093
Urine Hcy						-	0.133	0.137
Plasma Cys							…	0.695[a]
CKD Stages III–V								
eGFR	…	–0.019	0.346	0.046	–0.280	–0.131	–0.334[a]	–0.595[b]
Urine Crn		…	0.384	0.021	0.217	0.444	0.071	0.139
Urine SAM			…	0.247	0.108	0.031	–0.101	–0.043
Urine SAH				…	–0.030	–0.021	–0.105	0.068
Urine Cys					…	0.570[a]	0.021	0.121
Urine Hcy						…	0.066	0.144
Plasma Cys							…	0.654[b]

eGFR, estimated glomerular filtration rate; Crn, creatinine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Cys, cysteine; Hcy, homocysteine; CKD, chronic kidney disease.
[a]Corrected P <.001.
[b]P <.001.

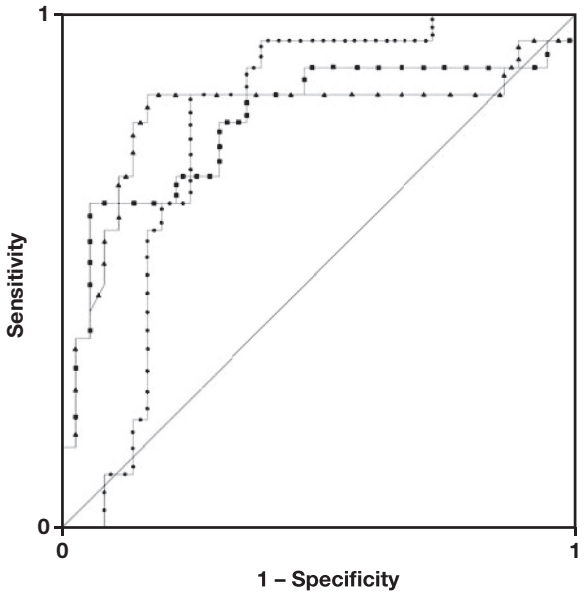
is already occurring, even despite the activation of cystathionine-β-synthase due to an increase in the level of SAM in blood plasma.

The results of a recent study[41] show that, at HHcy, expression of another transsulfuration enzyme, cystathionine-γ-lyase, is inhibited by a mechanism associated with the epigenetic regulation of transcription of these genes (ie, DNA methylation). We emphasize here that in addition to the utilization of Hcy, these enzymes perform the function of synthesizing hydrogen sulfide, which plays an important role not only in maintaining endothelial function but also in regulating glomerular filtration, promoting sodium reabsorption, and protecting the kidneys from damaging factors.[42] Therefore, impaired hydrogen sulfide metabolism is now considered to be a key mechanism of Hcy nephrotoxicity in CKD and a promising therapeutic target.

In addition to HHcy, patients with CKD are characterized by an increase in the plasma level of its predecessors—SAM and, in particular, SAH.[39,43,44] We emphasize that the association of plasma SAH and the SAM/SAH ratio with the stages of CKD in patients with type 2 diabetes was previously reported.[39] Mean levels of SAM and SAH at CKD stages II and V were 80 and 162 nM, and 10.5 and 112.7 nM, respectively; likewise, the SAM/SAH ratio decreased from 6.3 at stage II disease to approximately 1.5 at stage V. However, significant differences were observed only between CKD stage II and stages IV and V (SAH) and between CKD stages II and V (SAM and SAM/SAH).

Due to the close metabolic relationship of SAM with methylation and renal function, the accumulation of plasma SAM in patients with CKD may be explained by the global suppression of transmethylation reactions by SAH because SAH is a potent inhibitor of intracellular methylation reactions,[39] as well as the instigator of a decrease in eGFR. However, in contrast, it cannot be ruled out that some methylation reactions are intensified, including the formation of creatine, a Crn precursor that requires SAM. There has also been evidence[23,45] of suppression of a number of genes by uremic toxins realized through DNA methylation;

Lab Medicine 2021;52;47–56 53
DOI: 10.1093/labmed/lmaa034



	AUC	CI 95%	p
SAM/SAH urine	0.799	0.647-0.950	0.0003
SAM urine	0.775	0.648-0.902	0.0009
Cys/Hcy plasma	0.789	0.645-0.934	0.0005

Figure 2

Receiver operating characteristic (ROC) curves for plasma cysteine (Cys)/ homocysteine (Hcy), urine S-adenosylmethionine (SAM) and SAM/S-adenosylhomocysteine (SAH); CKD stage II vs III–V chronic kidney disease (CKD) stages.

also, the role of DNA hypermethylation in renal fibrosis was shown.

Unlike amino acids, SAM and SAH are much more poorly reabsorbed,[30] and their level in urine is 30 to 100 times higher than in blood plasma, which greatly facilitates their concentration in urine, compared with plasma.[30,46] It was previously found[29] that in patients with CKD, the levels of SAM and SAM/SAH in the urine were 3.1 μM and 3.41 μM, respectively, which was significantly lower than in healthy volunteers, in whom these levels were 10.2 μM and 11 μM, respectively.

In this pilot study, we first determined urine SAM and SAH levels in patients with CKD at different stages and examined the relationships between these indicators and eGFR, as well as the levels of Hcy and Cys in blood plasma. The main result is that a significant decrease in SAM and SAM/SAH ratios in urine was detected in patients with CKD at stages III through V, compared with patients with CKD at stage II.

We did not, however, reveal a correlation of eGFR with plasma HHcy and SAM urine levels in patients with stage II CKD; however, such correlations were observed in the entire range of patients with CKD at stages II through V. This finding can hardly be explained by a narrow range of the aforementioned indicators in this group of patients. Apparently, a slight decrease in renal function simply does not have a dominant effect on the levels of circulating HHcy and SAM excretion, among other factors (genetic, dietary, lifestyle, concomitant diseases, etc). However, in this group, there was a strong association of SAM and SAH levels in urine, which was absent in the later stages of CKD; this finding was previously shown[46] to be characteristic of healthy patients.

In contrast, significant changes in urine SAM, SAM/SAH, and plasma Cys/Hcy between stages III and V of CKD were not detected, despite an increase in the level of Crn in blood plasma. This finding may be due to the fact that we did not find a reliable correlation of eGFR with the level of SAM in urine, despite the good results of the ROC analysis. The AUC for these noninvasive indicators was close to 0.8 when classifying patient disease at stage II and III through V of CKD, which was close to the AUC for the Cys/Hcy ratio and higher than the AUC for Hcy in plasma. Despite the fact that the level of Hcy in plasma is closely associated with the development of CKD, our results suggest that the levels of SAM or SAM/SAH in urine are better qualifiers for the progression of CKD at its initial stages than the level of plasma Hcy.

Thus, the transition from mild to moderate CKD has the most pronounced effect on SAM excretion, which indicates that the level of SAM in urine can serve as an indicator of this transition. We also found that in these patients with CKD, there was not a reliable association between urine SAM level and eGFR, and urine Crn level. All these findings indicate a strong change in the excretion of SAM and SAH in the context of moderate and severe CKD, which can hardly be explained by a simple decrease in the concentration function of the kidneys.

Conclusions

Urine SAM testing is of interest as a noninvasive form of testing to determine the presence or absence of an

indicator of mild to moderate renal impairment. The results indicate the need for more detailed studies of SAM excretion in CKD, as well as the possibility of using urine SAM testing to monitor the assessment and prognosis of CKD. **LM**

Acknowledgments

This work was supported by the Russian Science Foundation (grant no. 16-15-10340).

References

1. Levey AS, Coresh J. Chronic kidney disease. *Lancet.* 2012;379(9811):165–180.
2. Lopez-Giacoman S, Madero M. Biomarkers in chronic kidney disease, from kidney function to kidney damage. *World J Nephrol.* 2015;4(1):57–73.
3. Nowak N, Skupien J, Smiles AM, et al. Markers of early progressive renal decline in type 2 diabetes suggest different implications for etiological studies and prognostic tests development. *Kidney Int.* 2018;93(5):1198–1206.
4. Wasung ME, Chawla LS, Madero M. Biomarkers of renal function, which and when? *Clin Chim Acta.* 2015;438:350–357.
5. Kumagai T, Ota T, Tamura Y, et al. Time to target uric acid to retard CKD progression. *Clin Exp Nephrol.* 2017;21(2):182–192.
6. Guessous I, Ponte B, Marques-Vidal P, et al. Clinical and biological determinants of kidney outcomes in a population-based cohort study. *Kidney Blood Press Res.* 2014;39(1):74–85.
7. Garlo KG, White WB, Bakris GL, et al. Kidney biomarkers and decline in eGFR in patients with type 2 diabetes. *Clin J Am Soc Nephrol.* 2018;13(3):398–405.
8. Penno G, Solini A, Bonora E, et al. Clinical significance of nonalbuminuric renal impairment in type 2 diabetes. *J Hypertens.* 2011;29(9):1802–1809.
9. Li A, Yi B, Liu Y, et al. Urinary NGAL and RBP are biomarkers of normoalbuminuric renal insufficiency in type 2 diabetes mellitus. *J Immunol Res.* 2019;2019:5063089.
10. Dincer Y, Akkaya C, Alagoz S, Pekpak M. Assessment of urinary epidermal growth factor level in patients with chronic kidney disease. *Urol Nephrol Open Access J.* 2018;6(4):131–134.
11. Hagiyama M, Nakatani Y, Takashima Y, et al. Urinary cell adhesion molecule 1 is a novel biomarker that links tubulointerstitial damage to glomerular filtration rates in chronic kidney disease. *Front Cell Dev Biol.* 2019;7:111.
12. Good DM, Zürbig P, Argilés A, et al. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. *Mol Cell Proteomics.* 2010;9(11):2424–2437.
13. Schanstra JP, Zürbig P, Alkhalaf A, et al. Diagnosis and prediction of CKD progression by assessment of urinary peptides. *J Am Soc Nephrol.* 2015;26(8):1999–2010.
14. Siwy J, Zürbig P, Argiles A, et al. Noninvasive diagnosis of chronic kidney diseases using urinary proteome analysis. *Nephrol Dial Transplant.* 2017;32(12):2079–2089.
15. Pontillo C, Jacobs L, Staessen JA, et al. A urinary proteome-based classifier for the early detection of decline in glomerular filtration. *Nephrol Dial Transplant.* 2017;32(9):1510–1516.
16. Zürbig P, Jerums G, Hovind P, et al. Urinary proteomics for early diagnosis in diabetic nephropathy. *Diabetes.* 2012;61(12):3304–3313.
17. Vivekanandan-Giri A, Slocum JL, Buller CL, et al. Urine glycoprotein profile reveals novel markers for chronic kidney disease. *Int J Proteomics.* 2011;2011:214715.
18. Lyu L-L, Feng Y, Liu B-C. Urinary biomarkers for chronic kidney disease with a focus on gene transcript. *Chin Med J (Engl).* 2017;130(18):2251–2256.
19. Saulnier P-J, Darshi M, Wheelock KM, et al. Urine metabolites are associated with glomerular lesions in type 2 diabetes. *Metabolomics.* 2018;14(6):84.
20. Maciejczyk M, Szulimowska J, Taranta-Janusz K, et al. Salivary FRAP as a marker of chronic kidney disease progression in children. *Antioxidants (Basel).* 2019;8(9):E409.
21. Smyth LJ, McKay GJ, Maxwell AP, McKnight AJ. DNA hypermethylation and DNA hypomethylation is present at different loci in chronic kidney disease. *Epigenetics.* 2014;9(3):366–376.
22. Wing MR, Devaney JM, Joffe MM, et al. DNA methylation profile associated with rapid decline in kidney function: findings from the CRIC study. *Nephrol Dial Transplant.* 2014;29(4):864–872.
23. Onishi A, Sugiyama H, Kitagawa M, et al. Urine 5MedC, a marker of DNA methylation, in the progression of chronic kidney disease. *Dis Markers.* 2019;2019:5432453.
24. Perna AF, Ingrosso D, Satta E, et al. Homocysteine metabolism in renal failure. *Curr Opin Clin Nutr Metab Care.* 2004;7(1):53–57.
25. Levi A, Cohen E, Levi M, et al. Elevated serum homocysteine is a predictor of accelerated decline in renal function and chronic kidney disease: a historical prospective study. *Eur J Intern Med.* 2014;25(10):951–955.
26. Xie D, Yuan Y, Guo J, et al. Hyperhomocysteinemia predicts renal function decline: a prospective study in hypertensive adults. *Sci Rep.* 2015;5:16268.
27. Garibotto G, Sofia A, Saffioti S, et al. Amino acid and protein metabolism in the human kidney and in patients with chronic kidney disease. *Clin Nutr.* 2010;29(4):424–433.
28. Guttormsen AB, Ueland PM, Svarstad E, Refsum H. Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. *Kidney Int.* 1997;52(2):495–502.
29. Kruglova MP, Grachev SV, Bulgakova PO, et al. Low S-adenosylmethionine/ S-adenosylhomocysteine ratio in urine is associated with chronic kidney disease. *Lab Med.* 2020;51(1):80–85.
30. Garibotto G, Valli A, Anderstam B, et al. The kidney is the major site of S-adenosylhomocysteine disposal in humans. *Kidney Int.* 2009;76(3):293–296.
31. Levey AS, Coresh J; National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. *Am J Kidney Dis.* 2002;39(Suppl 1):1–266.
32. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604–612.
33. Ivanov AV, Virus ED, Luzyanin BP, Kubatiev AA. Capillary electrophoresis coupled with 1,1'-thiocarbonyldiimidazole derivatization for the rapid detection of total homocysteine and cysteine in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2015;1004:30–36.
34. Ivanov AV, Bulgakova PO, Virus ED, et al. Capillary electrophoresis coupled with chloroform-acetonitrile extraction for rapid and highly selective determination of cysteine and homocysteine levels in human blood plasma and urine. *Electrophoresis.* 2017;38(20):2646–2653.

35. Ivanov AV, Virus ED, Nikiforova KA, et al. Capillary electrophoresis and phenylboronic acid solid phase extraction for the determination of S-adenosylmethionine/S-adenosylhomocysteine ratio in human urine. *Electrophoresis.* 2016;37(20):2663–2669.

36. Liotta E, Gottardo R, Bonizzato L, et al. Rapid and direct determination of creatinine in urine using capillary zone electrophoresis. *Clin Chim Acta.* 2009;409(1–2):52–55.

37. Ponte B, Pruijm M, Marques-Vidal P, et al. Determinants and burden of chronic kidney disease in the population-based CoLaus study: a cross-sectional analysis. *Nephrol Dial Transplant.* 2013;28(9): 2329–2339.

38. Fox CS, Gona P, Larson MG, et al. A multi-marker approach to predict incident CKD and microalbuminuria. *J Am Soc Nephrol.* 2010;21(12):2143–2149.

39. Herrmann W, Schorr H, Obeid R, et al. Disturbed homocysteine and methionine cycle intermediates S-adenosylhomocysteine and S-adenosylmethionine are related to degree of renal insufficiency in type 2 diabetes. *Clin Chem.* 2005;51(5):891–897.

40. Pastore A, Noce A, Di Giovamberardino G, et al. Homocysteine, cysteine, folate and vitamin B_{12} status in type 2 diabetic patients with chronic kidney disease. *J Nephrol.* 2015;28(5):571–576.

41. Li J-J, Li Q, Du H-P, et al. Homocysteine triggers inflammatory responses in macrophages through inhibiting CSE-H2S signaling via DNA hypermethylation of CSE promoter. *Int J Mol Sci.* 2015;16(6):12560–12577.

42. Feliers D, Lee HJ, Kasinath BS. Hydrogen sulfide in renal physiology and disease. *Antioxid Redox Signal.* 2016;25(13):720–731.

43. Loehrer FM, Angst CP, Brunner FP, Haefeli WE, Fowler B. Evidence for disturbed S-adenosylmethionine: S-adenosylhomocysteine ratio in patients with end-stage renal failure: a cause for disturbed methylation reactions? *Nephrol Dial Transplant.* 1998;13(3):656–661.

44. Valli A, Carrero JJ, Qureshi AR, et al. Elevated serum levels of S-adenosylhomocysteine, but not homocysteine, are associated with cardiovascular disease in stage 5 chronic kidney disease patients. *Clin Chim Acta.* 2008;395(1–2):106–110.

45. Bechtel W, McGoohan S, Zeisberg EM, et al. Methylation determines fibroblast activation and fibrogenesis in the kidney. *Nat Med.* 2010;16(5):544–550.

46. Stabler SP, Allen RH. Quantification of serum and urinary S-adenosylmethionine and S-adenosylhomocysteine by stable-isotope-dilution liquid chromatography-mass spectrometry. *Clin Chem.* 2004;50(2):365–372.

Associations of Serum Pepsinogens and *Helicobacter Pylori* Infection with High-Sensitivity C-Reactive Protein in Medical Examination Population

Huixing Liu, MS,[1] *Chunrong Fei, BS,*[1] *Jun Zhang, PhD*[1*]

Laboratory Medicine 2021;52:57-63

DOI: 10.1093/labmed/lmaa042

ABSTRACT

Objective: This study aimed to clarify the distribution characteristics of serum pepsinogen (PG) and *Helicobacter pylori* in the medical examination population and to explore the relationships of PG level and *H. pylori* infection status with the high-sensitivity C-reactive protein (hsCRP) level and their significance in health examination.

Methods: We detected *H. pylori* infection by C^{13} urea breath test, the serum pepsinogen I (PGI) and pepsinogen II (PGII) contents were measured by chemiluminescence microparticle immunoassay, and the PGI/PGII ratio was calculated. In addition, the serum hsCRP level was determined by the Abbott C16000 automatic biochemical analyzer.

Results: The PGI and hsCRP levels were significantly higher in men than in women, and the PGII level was slightly higher in men than in women (both $P < .05$). The PGI, PGII, and hsCRP levels were positively correlated with age ($r = 0.210$, 0.287, and 0.133, respectively; $P < .05$), whereas the PGI/PGII ratio was negatively correlated with age ($r = -0.190$; $P < .05$). The positive *H. pylori* infection rate was 30.2% among the patients in this study; *H. pylori* infection was not related to sex ($P > .05$), and the difference in age stratification was not statistically significant ($P > .05$). The abnormal PGI/PGII ratio in the medical examination population was not correlated with sex ($P > .05$). In the *H. pylori* positive infection group, the proportion of PGI/PGII ratio <3, the PGI and PGII levels were significantly higher than those in the *H. pylori* negative infection group, and the PGI/PGII ratio was significantly lower than that in the negative group (both $P < .05$). The hsCRP level was not associated with *H. pylori* infection ($P > .05$), and it was significantly higher in the PGI/PGII ratio <3 group than in the PGI/PGII ratio ≥3 group ($P < .05$).

Conclusion: The PGI and PGII levels and the PGI/PGII ratio are correlated with *H. pylori* infection. The abnormal PGI/PGII ratio is closely related to *H. pylori* infection and hsCRP level. Therefore, *H. pylori* infection status and hsCRP level should be considered when determining atrophic gastritis by the PGI/PGII ratio.

Keywords: medical examination, *Helicobacter pylori* infection, high-sensitivity C-reactive protein, pepsinogen I, pepsinogen II, gastric cancer

Gastric cancer (GC), a frequently occurring gastrointestinal tract malignancy, has high morbidity and mortality.[1] Gastroscopy is the gold standard for diagnosing GC, but it cannot be used as a suitable screening program in health examination because of its invasiveness. Pepsinogens (PGs) are the inactive precursors of pepsins in gastric juice and are classified immunologically into 2 types: pepsinogen I (PGI) and pepsinogen II (PGII). The first type, PGI, is secreted by the fundic gland, and PGII is released by the fundic, cardiac, pyloric, and Brunner glands. In the case of fundic gland mucosal atrophy, the number of PGI-secreting gastric chief cells decreases and that of pyloric gland cells increases, thereby leading to a decreased PGI/PGII ratio.[2–4] Research has shown that GC is related to fundic gland mucosal atrophy–associated diseases, especially atrophic gastritis. Hence, the immunological determination of PGs is applicable to GC screening.[5–7] High-sensitivity C-reactive protein (hsCRP) is an acute phase-reactive protein that is nonspecifically upregulated upon inflammatory response in organisms. According to previous studies,[8,9] hsCRP is valuable in tumor screening. Studies have shown that GC is associated with inflammation and hsCRP is upregulated by the cytokines interleukin-6 (IL-6), IL-8, and tumor necrosis

Abbreviations:

PG, pepsinogen; hsCRP, high-sensitivity C-reactive protein; PGI, pepsinogen I; PGII, pepsinogen II; GC, gastric cancer; IL, interleukin.

[1]Clinical Laboratory, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China

*To whom correspondence should be addressed.
jameszhang2000@zju.edu.cn

factors during gastric mucosal tissue carcinogenesis.[8,10–12] As a high-risk factor for GC, *Helicobacter pylori* infection increases the risk of GC in patients with chronic atrophic gastritis.[13] Based on previous studies,[8,14] *H. pylori* infection is correlated with serum PG levels. To this end, this study aimed to explore the associations of serum PG and hsCRP levels with *H. pylori* infection and to analyze their significance in health examination.

Materials and Methods

Patients treated in 2019 at the Physical Examination Center of Sir Run Run Shaw Hospital affiliated with the Zhejiang University School of Medicine who received C^{13} urea breath, PG, and hsCRP tests simultaneously were selected as the participants in this retrospective analysis. The patient exclusion criteria were as follows: patients who took antacids, gastric mucosal protectants, and antibiotics in the past 1 month; those with heart, liver, or kidney insufficiency; those with gastropathy; and those with acute or chronic inflammatory diseases. Finally, 643 patients were enrolled in this study, including 375 men and 268 women, aged 17 to 87 years (mean, 50.43 ± 13.50).

Five mL fasting venous blood was sampled from each patient, then serum was separated to determine the PGI and PGII levels, calculate the PGI/PGII ratio, and measure the hsCRP level. An existing *H. pylori* infection was detected by a C^{13} urea breath test completed in the endoscopy room of the gastroenterology department.

PG Determination and Analysis

The chemiluminescent microparticle immunoassay kit (Abbott Laboratories) in the ARCHITECT i2000 automated immunoassay analyzer was used to determine the PG level. In the clinical setting, a PGI/PGII ratio <3 was used as the threshold to evaluate the severity of fundic gland mucosal atrophy.

Determination and Analysis of hsCRP

Latex particle-enhanced turbidimetric immunoassay detected hsCRP levels using the Abbott C16000 automated biochemical analyzer and supporting reagents. In the clinical setting, an hsCRP level ≤5 mg/L was taken as the reference range.

The C^{13} Urea Breath Test

The HCBT-01 breath test analyzer detected *H. pylori* using the Urea-[13C] Capsule Breath Test Kit (Shenzhen Zhonghe Headway Bio Science & Technology Co, Ltd).

Statistical Analysis

SPSS 19.0 was utilized for statistical analysis, and data were expressed as mean ± standard deviation (X ± SD). The normally distributed measurement data were analyzed by the Student's *t*-test and the Mann-Whitney *U* test was used for pairwise comparison for skewed-distributed measurement data. The Kruskal-Wallis *H* test was conducted for comparisons among multiple groups. In the meantime, intergroup comparisons were expressed as percentages and analyzed by χ^2 test, while the Spearman technique was used for correlation analysis. A difference of $P < .05$ indicated statistical significance.

Results

Associations of Serum PG and hsCRP Levels with Sex

For the 643 patients (including 375 men and 268 women), the serum PGI, PGII, PGI/PGII ratio, and hsCRP levels are shown in **Figure 1**. Male patients exhibited higher PGI and hsCRP levels than female patients and slightly higher PGII levels as well ($P < .05$). Meanwhile, there was no significant difference in the PGI/PGII ratio between men and women ($P > .05$).

Relationships of Serum PG and hsCRP Levels with Age

Patients were divided into 5 age groups in 10-year increments: aged <40 years (n = 139), 40 to 49 years (n = 180), 50 to 59 years (n = 151), 60 to 69 years (n = 114), and ≥70 years (n = 59). Relevant results are presented in **Table 1**. Serum PGI, PGII, and hsCRP levels increased accordingly with age. There was

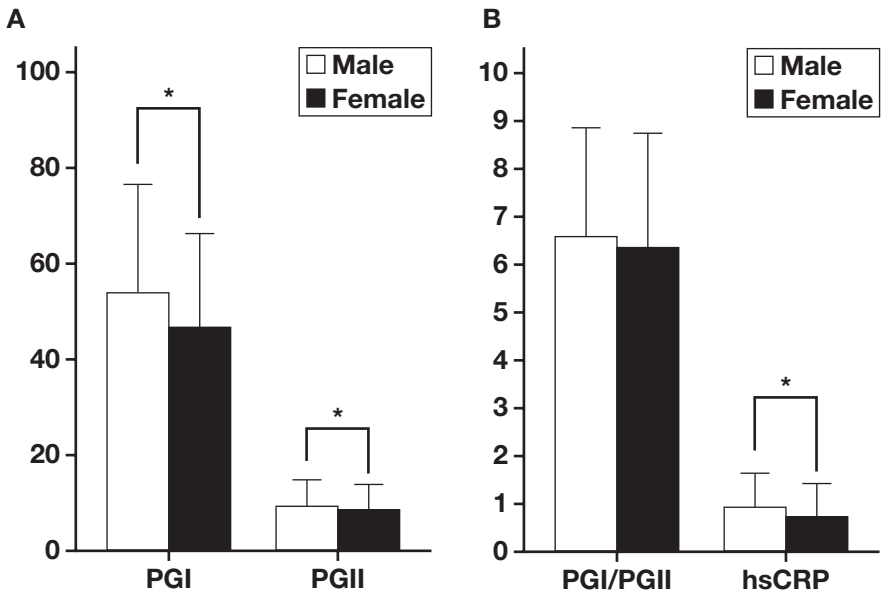


Figure 1

Associations of serum PG and hsCRP levels with sex.

Table 1. Interage Differences in Serum PG and hsCR Levels

Group (patients, age, y)	PGI (ng/mL)	PGII (ng/mL)	PGI/PGII Ratio	hsCRP (mg/L)
<40 (139)	45.49 ± 17.55	7.20 ± 4.54	7.07 ± 2.06	0.82 ± 0.78
40–49 (180)	47.44 ± 17.72	7.96 ± 5.34	6.92 ± 2.27	0.83 ± 0.65
50–59 (151)	54.22 ± 21.65	9.98 ± 5.75	6.21 ± 2.18	0.85 ± 0.57
60–69 (114)	55.53 ± 21.33	10.12 ± 6.25	6.34 ± 2.36	0.92 ± 0.65
≥70 (59)	55.13 ± 21.65	10.89 ± 6.29	5.69 ± 2.79	1.05 ± 0.99

no difference in serum PGI level between the 60- to 69-year-old and ≥70-year-old groups, but the PGI/PGII ratios were markedly lower in the 50- to 59-year-old and ≥70-year-old groups than those in the other groups. As suggested by the Kruskal-Wallis *H* test of multiple independent samples, the differences in the serum PGI, PGII, PGI/PGII ratio, and hsCRP levels were statistically significant between various age groups (P <.05).

Correlations of Serum PG and hsCRP Levels with Age

As shown in **Figure 2A**, **Figure 2B**, and **Figure 2D**, serum PGI, PGII, and hsCRP levels were positively correlated with age (P <.05), and the PGI/PGII ratio was negatively correlated with age (P <.05) (**Figure 2C**).

Associations of *H. pylori* Positive Rate with Age and Sex

Results showed that 194 of the 643 patients were *H. pylori* positive, resulting in a positive rate of 30.2% (**Table 2** and **Figure 3**).

Associations of Serum PGI, PGII, PGI/PGII Ratio, and hsCRP Levels with *H. pylori* Infection

We found that patients who were *H. pylori* positive exhibited significantly higher PGI and PGII levels than those who were *H. pylori* negative (P <.05). Meanwhile, patients who were *H. pylori* positive had a significantly lower PGI/PGII ratio than those who were *H. pylori* negative (P <.05). Compared with the PGI level, the PGII level in patients who were *H. pylori* positive dramatically increased. In addition,

Lab Medicine 2021;52;57–63
DOI: 10.1093/labmed/lmaa042

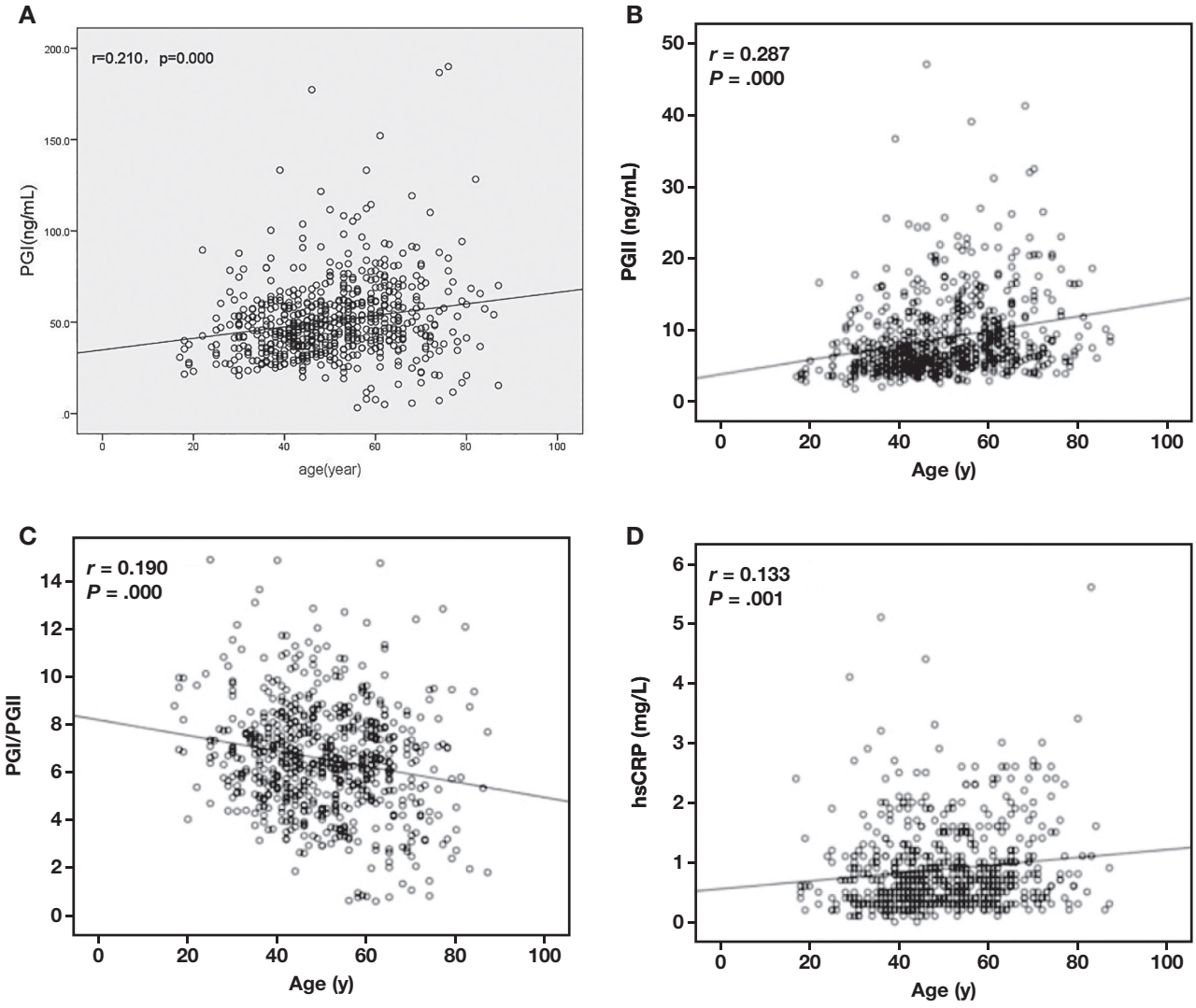


Figure 2

Correlations of serum PG and hsCRP levels with age. **A**, Correlation between serum PGI level and age. **B**, Correlation between serum PGII level and age. **C**, Correlation between PGI/PGII ratio and age. **D**, Correlation between serum hsCRP level and age.

there was no difference in hsCRP level between patients who were *H. pylori* positive and *H. pylori* negative ($P > .05$) (**Table 3**).

Associations of PGI/PGII Abnormality with Sex and *H. pylori* Infection

Among the population in this study, 34 patients had a PGI/PGII ratio <3, yielding the abnormal rate of 5.29%. According to our results, PGI/PGII abnormality was correlated with *H. pylori* infection but not with sex. In addition, there were significantly more patients with a PGI/PGII ratio <3 in among those who were *H. pylori* positive than *H. pylori* negative (10.82% vs. 2.90%, $\chi^2 = 17.008$; $P < .05$). **Table 4** lists the relevant results.

Changes in hsCRP Level in Patients with Abnormal PGI/PGII Ratio

The hsCRP level was higher in patients with a PGI/PGII ratio <3 than in those with a PGI/PGII ratio ≥3 ($P < .05$) (**Table 5**).

Table 2. Association of *H. pylori* Positive Rate with Gender

Group (patients)	*H. pylori* Positive Rate (%)	Patients *H. pylori* Positive
Total (643)	30.2	194
Male (375)	31.7	119
Female (268)	28.0	75
Patients by age (y)		
<40 (139)	28.8	40
40–49 (180)	28.9	52
50–59 (151)	32.5	49
60–69 (114)	28.1	32
≥70 (59)	35.6	21

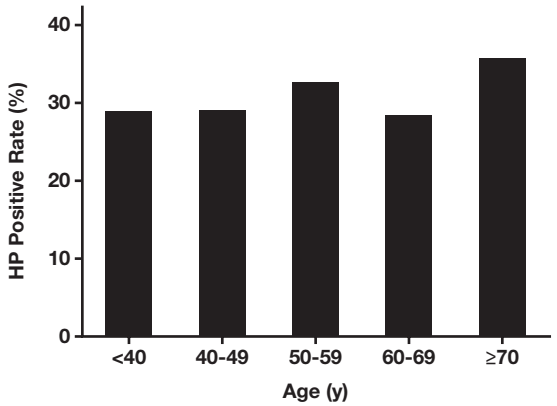


Figure 3

Associations of *H. pylori* positive rate with age.

Discussion

This study suggested that PGI and PGII levels were associated with sex (levels were higher in men than in women) and positively correlated with age. These results are consistent with other studies.[14,15] In addition, our results showed that patients who were *H. pylori* positive had markedly higher PGI and PGII levels than those who were *H. pylori* negative. Possible reasons include the following: (i) *H. pylori* lipopolysaccharides participate in PG secretion;[15] (ii) *H. pylori* increases the cyclic adenosine monophosphate level by stimulating calcium ion mobilization in gastric chief cells and activating adenylate cyclase, thereby promoting PG secretion;[16] and (iii)) ILs and tumor necrosis factors induce the enhanced secretion of gastrin and gastric acids to stimulate PG secretion.[14]

In addition, this study also suggested that the PGII level increased more drastically than the PGI level for patients who were *H. pylori* positive. Other researchers[17,18] also showed that the serum PGII level increases significantly in patients infected with *H. pylori*, stating that a high serum PGII level predicts *H. pylori*–induced gastritis, which is mainly the result of neutrophil and monocyte infiltration. In fact, existing studies confirm that PGII declines following *H. pylori* eradication, arguing that PGII level serves as an indicator for *H. pylori* eradication.[14,19]

Research has shown that GC develops in the process from chronic inflammation to glandular atrophy to intestinal metaplasia to atypical hyperplasia to carcinogenesis.[1,8] Atrophic gastritis is considered to be a precancerous lesion of GC. As shown in previous studies,[2,8] the PGI/PGII ratio is a superior indicator for the early screening and assessment of GC metastatic status, and a smaller PGI/PGII ratio implies a greater risk of GC. In this study, the PGI/PGII ratio was negatively correlated with age but not with sex. In clinical practice, a PGI/PGII ratio <3 is used as the threshold to evaluate the severity of fundic gland mucosal atrophy. As confirmed by Su and Zhou[1] and Nardone et al,[20] the sensitivity and specificity of using a PGI/PGII ratio <3 as a threshold to detect atrophic gastritis are 71% and 86%, respectively. In line with this standard, the morbidity of atrophic gastritis was 5.29% in the patients participating in this study. As revealed by our results, PGI/PGII abnormality was not related to sex, but the PGI/PGII level significantly decreased in patients who were *H. pylori* positive, and the proportion of patients with a PGI/PGII ratio <3 increased significantly. As observed from **Table 1** and **Figure 3**, the 50- to 59-year-old and ≥70-year-old age groups had lower PGI/PGII levels and higher *H. pylori* infection rates accordingly. In addition, the *H. pylori* positive rate was prominently higher in those with a PGI/PGII ratio <3 than in those with a PGI/PGII ratio ≥3 (results not shown). Thus, *H. pylori* infection may be closely linked to PGI/PGII abnormality. In future studies, more attention should be paid to patients who are *H. pylori* positive with a PGI/PGII ratio <3.

In addition, hsCRP is an acute phase-reactive protein produced upon inflammation, trauma, or infection stimulation in organisms. An elevated hsCRP level has also been reported in some noninfectious diseases,[21] and increasing attention is paid to its value in tumor screening.[8,9,12] In this study, hsCRP levels in patients with a PGI/PGII ratio <3 were higher than in those with a PGI/PGII ratio ≥3. Yang and Chen[21] also showed

DOI: 10.1093/labmed/lmaa042

Table 3. Associations of Serum PGI, PGII, PGI/PGII Ratio, and hsCRP Levels with *H. pylori* Infection

	PGI (ng/mL)	PGII (ng/mL)	PGI/PGII Ratio	hsCRP (mg/L)
H. pylori–positive group	59.79 ± 23.14	13.69 ± 6.86	4.87 ± 1.76	0.94 ± 0.74
H. pylori–negative group	46.84 ± 19.86	6.87 ± 3.47	7.31 ± 2.13	0.86 ± 0.70
Z value	–7.864	–14.242	–13.480	–1.671
P value	<.05	<.05	<.05	>.05

Table 4. Associations of PGI/PGII Abnormality with Gender and *H. pylori* Infection

	n	PGI/PGII Ratio ≥3	PGI/PGII Ratio <3
Total	643	609	34 (5.29%)
Male	375	356	19 (5.07%)
Female	268	253	15 (5.60%)
H. pylori–positive	194	173	21 (10.82%)[a]
H. pylori–negative	449	436	13 (2.90%)

[a]P *<.05 vs* H. pylori–*negative group.*

Table 5. Association of hsCRP Level with PGI/PGII Ratio

	n	hsCRP (mg/L)
PGI/PGII ratio ≥3	609	0.85 ± 0.67
PGI/PGII ratio <3	34	1.12 ± 1.02
P value		<.05

higher hsCRP levels in patients with atrophic gastritis than in control patients. Moreover, hsCRP levels were positively correlated with age and sex (higher in men than in women), but not with *H. pylori* infection. Chung et al[8] indicated that the diagnostic sensitivity and specificity of hsCRP plus the PGI/PGII ratio were up to 81% and 73% for advanced GC and that the combination of these 2 levels had a vital value in screening for GC among high-risk patients.

For the population studied herein, the *H. pylori* positive rate was 30.2% and was independent of age and sex, which was consistent with the Xu et al[22] report. Nevertheless, the *H. pylori* positive rate herein was lower than those reported by other researchers.[14,22,23] The following limitations should be noted in this study. First, our patients were physical examinees, and they did not represent the overall Chinese population. Second, participants who had regular physical examinations might have been in a higher economic demographic than the general Chinese population. Third, because of the retrospective study design, the information about the use of antibiotic drugs may not have been recorded clearly, and those patients who took antibiotic drugs were not excluded totally. Therefore, more comprehensive data are needed to grasp the *H. pylori* distribution across the general Chinese population.

Conclusion

In conclusion, in this study, serum PG is correlated with age, sex, and *H. pylori* infection, and a PGI/PGII abnormality indicates a higher *H. pylori* infection rate. Meanwhile, hsCRP levels also exhibit distinct disparities when stratified by age, sex and PGI/PGII abnormality. Thus, for physical examinees, especially for elderly men, more attention should be paid to gastric function examination. When diagnosing atrophic gastritis based on the PGI/PGII ratio, clinicians should adopt *H. pylori* infection status in combination with hsCRP levels. The combination of the PGI/PGII ratio, *H. pylori* infection, and hsCRP indexes can be used to detect gastropathy early in the medical examination patients. For those with abnormal results, gastroduodenoscopy can be performed to improve diagnostic and therapeutic efficiency. **LM**

References

1. Su WW, Zhou B, Qin GM, et al. Low PGI/PGII ratio as a marker of atrophic gastritis association with nutritional and metabolic status in healthy people. *Medicine*. 2018;97(20):1–7.
2. Liu X, Yu A, Liu Y, et al. Relationship between pepsinogen and other tumor markers and gastric cancer. *J Mod Lab Med*. 2018;33(5):108–111.
3. Correa P. Serum pepsinogens in gastric cancer screening. *Digest Dis Sci*. 2010;55(8):2123–2125.
4. Sun LP, Gong YH, Wang L, Yuan Y. Serum pepsinogen levels and their influencing factors: a population-based study in 6990 Chinese from North China. *World J Gastroenterol.* 2007;13(48):6562–6567.

5. Dinis-Ribeiro M, Yamaki G, Miki K, Costa-Pereira A, Matsukawa M, Kurihara M. Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. *J Med Screen.* 2004;11(3):141–147.
6. Kitahara F, Kobayashi K, Sato T, Kojima Y, Araki T, Fujino MA. Accuracy of screening for gastric cancer using serum pepsinogen concentrations. *Gut.* 1999;44(5):693–697.
7. Yoshihara M, Sumii K, Haruma K, et al. Correlation of ratio of serum pepsinogen I and II with prevalence of gastric cancer and adenoma in Japanese subjects. *Am J Gastroenterol.* 1998;93(7):1090–1096.
8. Chung HW, Kim JW, Lee J-H, et al. Comparison of the validity of three biomarkers for gastric cancer screening: carcinoembryonic antigen, pepsinogens, and high sensitive C-reactive protein. *J Clin Gastroenterol*. 2009;43(1):19–26.
9. Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S; Japan Public Health Center-Based Prospective Study Group. Plasma C-reactive protein and risk of colorectal cancer in a nested case-control study: Japan Public Health Center-based prospective study. *Cancer Epidemiol Biomarkers Prev.* 2006;15(4):690–695.
10. Mirvish SS. The etiology of gastric cancer. Intragastric nitrosamide formation and other theories. *J Natl Cancer Inst.* 1983;71(3):629–647.
11. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process–First American Cancer Society Award lecture on cancer epidemiology and prevention. *Cancer Res.* 1992;52(24):6735–6740.
12. Coussens LM, Werb Z. Inflammation and cancer. *Nature.* 2002;420(6917):860–867.
13. Ohata H, Kitauchi S, Yoshimura N, et al. Progression of chronic atrophic gastritis associated with Helicobacter pylori infection increases risk of gastric cancer. *Int J Cancer.* 2004;109(1):138–143.
14. Huang RG, Xiao HL, Zhou B, et al. Serum pepsinogen levels are correlated with age, gender, and the level of H. pylori infection in healthy individuals. *Am J Med Sci.* 2016;352(5):481–486.
15. Young GO, Stemmet N, Lastovica AJ, et al. Helicobacter pylori lipopolysaccharide stimulates gastric mucosal pepsinogen secretion. *Aliment Pharmacol Ther*. 1992;6(2):169–177.
16. Jiang HX, Pu H, Huh NH, et al. Helicobacter pylori induces pepsinogen secretion by rat gastric cells in culture via a cAMP signal pathway. *Int J Mol Med*. 2001;7(6):625–629.
17. Wang W, Liu Y. Relationship of serum pepsinogen II and gastric disease in health examination population. *Chin J Health Manage*. 2018;12(5):453–456.
18. Gu JX, Zhu XH, Wu YF, Pan JY. Value of combined diagnosis of serum gastrin 17, pepsinogen and helicobacter pylori in elderly patients with gastric precancerous lesions. *Int J Lab Med.* 2018;39(17):2109–2112.
19. Massarrat S, Haj-Sheykholeslami A, Mohamadkhani A, et al. Pepsinogen II can be a potential surrogate marker of morphological changes in corpus before and after H. pylori eradication. *Biomed Res Int.* 2014;2014:1–7.
20. Nardone G, Rocco A, Staibano S, et al. Diagnostic accuracy of the serum profile of gastric mucosa in relation to histological and morphometric diagnosis of atrophy. *Aliment Pharmacol Ther.* 2005;22(11-12):1139–1146.
21. Yang YG, Chen Y. The level of PGI, PGII and CRP in elderly male patients with atrophic gastritis. *Chin J Gerontol*. 2014;34(3):788–789.
22. Xu C, Yan M, Sun Y, et al. Prevalence of helicobacter pylori infection and its relation with body mass index in a Chinese population. *Helicobacter.* 2014;19(6):437–442.
23. Xi X-Y. Study of correlation between pepsinogen levels and Helicobacter pylori infection among people taking physical examination in Shanghai J. *Jiangsu J Prey Med*. 2015;26(1):32–34.

DOI: 10.1093/labmed/lmaa042

The Diagnostic Performance of lncRNAs from Blood Specimens in Patients with Hepatocellular Carcinoma: A Meta-Analysis

Jing-Yi Huang, BS,[1] Si-Yu Wang, BSMed,[2] Yong Lin, PhD,[2] Huo-Chun Yi, MM,[2] Jian-Jun Niu, PhD[2]*

Laboratory Medicine 2021;52:64-73

DOI: 10.1093/labmed/lmaa050

ABSTRACT

Objective: Long noncoding RNAs (lncRNAs) are widely involved in the carcinogenesis and development of cancers. We conducted a meta-analysis to evaluate the diagnostic performance of lncRNAs in hepatocellular carcinoma (HCC).

Methods: After the inclusion and exclusion process, relevant information was extracted. Heterogeneity between studies was evaluated, and data synthesis was conducted by employing a bivariate random-effects model.

Results: In total, 20 eligible studies were enrolled. The pooled sensitivity and specificity were 0.86 (95% confidence interval [CI], 0.80–0.90) and 0.88 (95% CI, 0.82–0.92), respectively. The pooled positive likelihood ratio, pooled negative likelihood ratio, and pooled diagnostic odds ratio were 7.1 (95% CI, 4.9–10.2), 0.16 (95% CI, 0.11–0.23), and 44 (95% CI, 25–79), respectively. The results of the linear regression method and visual inspection of the Deeks funnel plot did not indicate significant publication bias.

Conclusion: Our meta-analysis suggested that lncRNAs have high diagnostic performance for HCC and have the potential for clinical application.

Keywords: long noncoding RNAs, hepatocellular carcinoma, diagnostic performance, meta-analysis, serum biomarkers, in vitro diagnosis

Hepatocellular carcinoma (HCC) is a common cancer that places a heavy economic burden on the healthcare system and has become a severe public health issue that needs to be addressed. Its known etiology involves chronic hepatitis B virus (HBV) infection, which is very common in mainland China;[1] chronic hepatitis C virus (HCV) infection;[2] and excessive alcohol consumption, which mainly contributes to the incidences and deaths from HCC in Western countries.[3] Recently, diabetes has been linked to the elevated risk of developing HCC, and a large-scale cohort study has confirmed that the application of a type 2 diabetes mellitus prevention diet can significantly reduce the HCC risk in both men and women.[4] Although rapid progress has been made in HCC prevention and intervention, such as the universal application of the HBV vaccine and health education on alcohol consumption and diet, HCC still holds a leading position in the incidence and prevalence of all kinds of cancers worldwide, especially in China[5] and Africa.[6] In addition, in the early stage of HCC, patients seldom show symptoms or manifestations because of the absence of neuron cells on the surface of the liver. Therefore, most patients with HCC are diagnosed in advanced stages, accompanied by nausea, jaundice, and other symptoms. A cohort study conducted in 2887 patients with HCC showed that the 5-year survival rate was only 19.5% after 14 years of observation.[7] Given that HCC has a high prevalence and poor prognosis, it has been generally acknowledged that the early diagnosis of HCC is essential in improving the clinical outcome of HCC.

Alpha-fetoprotein (AFP) has been used for the screening of HCC in clinical practice for decades because its level is low in healthy adults but can be elevated in the case of liver injuries,

Abbreviations:

lncRNA, long noncoding RNA; HCC, hepatocellular carcinoma; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; TP, true positive; FP, false positive; FN, false negative; TN, true negative; QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies 2; SROC, summary receiver operating characteristic; AUC, area under the curve; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; qRT-PCR, quantitative reverse-transcription polymerase chain reaction.

[1]Clinical Laboratory, Xiamen Branch of Zhongshan Hospital, Fudan University, Xiamen, Fujian Province, China, [2]Center of Clinical Laboratory, Zhongshan Hospital, Xiamen University, Xiamen, Fujian Province, China

*To whom correspondence should be addressed.
niujianjun62@163.com

including liver fibrosis, chronic HBV infection, and HCC.[8] Nevertheless, AFP has several defects that limit its diagnostic performance. First, its elevation in serum is not obvious in early-stage HCC. According to a retrospective study, the sensitivity of AFP in diagnosing early HCC was only 47.30%, and even when patients from all stages were taken into account, its sensitivity was 64.80%.[9] Second, the presence of normal AFP levels in patients with HCC is another problem in employing AFP as a biomarker. In a study that involved 1616 patients with HCC, the data suggested that 47.1% of them had normal AFP levels in serum specimens.[10] Based on these results, it can be concluded that AFP is not a reliable biomarker for the diagnosis of HCC, and finding novel and efficient biomarkers is imperative for the management of HCC.

Long noncoding RNAs (lncRNAs) have been intensively investigated in all aspects of diseases, especially cancers. They are small RNAs > 200 nucleotides in length and have no protein-coding function.[11] Through functional genomics studies, lncRNAs have been associated with a wide range of biological functions, such as the regulation of gene activation or inactivation by targeting different chromatin modification complexes,[12] and they interact with mRNA-stabilizing proteins to alter gene expression. Growing evidence has shown that lncRNAs are associated with the metastasis and prognosis of HCC. For instance, *MALAT-1* could be upregulated by the X protein of HBV, and as a result, *MALAT-1* further promotes the expression of several growth factors that facilitate the progression and metastasis of HCC.[13] Gao, Xu, et al[14] identified 6 differentially expressed lncRNAs in comparing HCC tissues and corresponding normal tissues and measured *SNHG1* in plasma specimens, suggesting that it has great potential to be a biomarker in HCC diagnosis. To date, many similar studies have been conducted to investigate whether lncRNAs can serve as biomarkers in HCC diagnosis; however, the results were not consistent. In the present study, we conducted a comprehensive meta-analysis to evaluate the diagnostic performance of lncRNAs in distinguishing patients with HCC from those without HCC.

Materials and Methods

Literature Search

We conducted a literature search in the PubMed and China National Knowledge Infrastructure databases on January 19, 2020 to identify potential studies that could be used in the meta-analysis. The search terms used for literature retrieval and abstracts were as follows: ("lncRNA" OR "long noncoding RNA") AND ("hepatocellular carcinoma" OR "liver cancer" OR "HCC"). The literature search was independently conducted by 2 investigators to minimize possible biases.

Inclusion and Exclusion Criteria

Any study was considered eligible for inclusion in our meta-analysis if the following criteria were met: (i) all patients with HCC had to be diagnosed by pathological or ultrasound examination; (ii) control patients had no history of any kind of cancer; (iii) the sensitivity, specificity, and number of patients with HCC and control patients could be found in the literature; and (iv) the detection of lncRNA was performed in blood specimens. The exclusion criteria were as follows: (i) review and meta-analysis articles, (ii) lncRNA measurement was conducted in tissue specimens, (iii) duplicate publications, and (iv) data that were necessary for meta-analysis could not be extracted by reviewing the articles or by calculation.

Data Extraction and Quality Assessment

The following information was extracted from the full texts and supplemental materials of each eligible study: last name of the first author, year of publication, country of residence of the study participants, number of patients with HCC and control patients, gene symbol of lncRNAs studied, and true positive (TP), false positive (FP), false negative (FN), and true negative (TN) values. If no direct data of the diagnostic parameters were available, the values of these parameters were calculated backward through the values of the sensitivity and specificity. The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool was used to evaluate the risk of bias of all included publications. The evaluation process was similarly carried out by the 2 investigators. In the case of disagreements, a third investigator was invited to resolve the issue.

Statistical Analysis

After data extraction, the relevant data on diagnostic performance, including TP, FP, FN, and TN values, were recorded in STATA software (Stata Corporation, College Station, TX) for further analysis. A bivariate random-effects model was applied to conduct quantitative synthesis. With this approach, the pooled diagnostic performance indicators were estimated, and forest plots were used to display the overall effects. A summary receiver operating

DOI: 10.1093/labmed/lmaa050

characteristic (SROC) curve was also generated to estimate the area under the curve (AUC) with an optimal cutoff value. Bivariate boxplots along with the qualitative *Q* test and the quantitative I^2 test were used to evaluate interstudy heterogeneity. Publication bias was assessed by using the linear regression method and visual inspection of the Deeks funnel plot. The Fagan nomogram was used to describe the pretest probability and posttest probability of lncRNA detection. Statistical analysis was conducted by using STATA software, and all tests were 2-sided. A *P* value <.05 was considered significant.

Results

Features of Eligible Studies and Quality Assessment

By conducting a comprehensive search of the literature, we identified a total of 420 articles. After reviewing the titles and abstracts, 396 articles were excluded because they did not discuss the diagnostic performance of lncRNAs in patients with HCC. The remaining 24 articles were subjected to full-text review to determine if they were eligible for meta-analysis. After excluding 3 articles, we included a final total of 20 papers in the present meta-analysis. The inclusion and exclusion processes are presented in the form of a flow diagram in **Figure 1**.

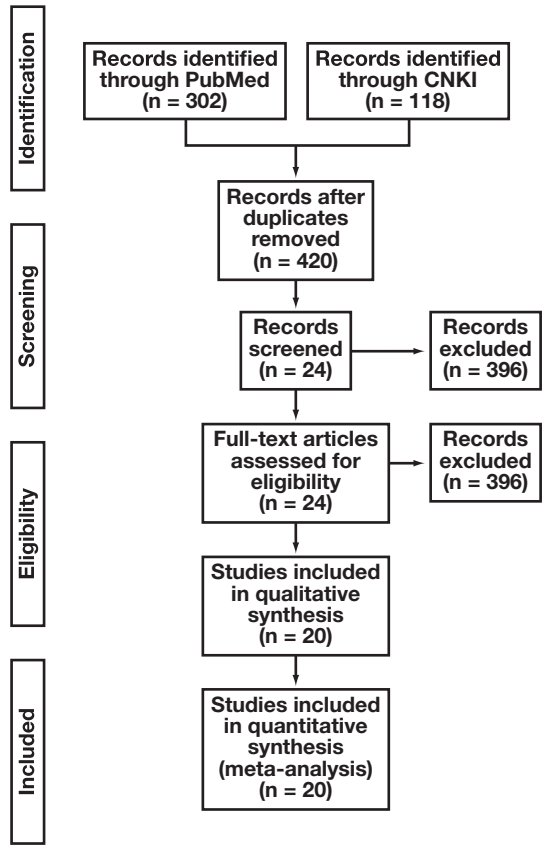


Figure 1

Flow diagram of the inclusion and exclusion process based on PRISMA.

The 20 selected studies in our meta-analysis included 1928 patients with HCC and 1897 control patients.[14-33] As seen in **Table 1**, all included studies were published between 2013 and 2019, because lncRNAs were only recently discovered. Regarding the country of residence, the majority of studies were conducted in China, 2 were conducted in Egypt, and 1 was conducted in Japan. We also calculated the average sample size for each study, and the average number of participants was approximately 191 people. In **Table 2**, the types of specimens, the anticoagulants used in specimen collection, and the detection methods of lncRNAs are listed. Half of the studies used serum as the material for lncRNA measurement and the other half employed plasma. Among the 10 studies that collected plasma specimens, 7 used EDTA as an anticoagulant and 3 did not specify the type of anticoagulant used. All included studies employed quantitative reverse-transcription polymerase chain reaction to measure the expression level of lncRNAs.

The QUADAS-2 tool has been widely applied in the quality evaluation of diagnostic studies and has 4 domains: patient selection, index test, reference standard, and flow and timing. Based on the results of the QUADAS-2 tool (**Figure 2**), the risk of bias in the study was low and the clinical applicability was moderate.

Diagnostic Performance

By statistical analysis, we found that both sensitivity and specificity had significant interstudy heterogeneity: the *P* values of the *Q* test for both parameters were < 0.001. Moreover, the bivariate boxplot shown in **Figure 3** also showed that 3 studies deviated from the center, indicating the existence of interstudy heterogeneity. Therefore, a

Table 1. The Basic Features of Included Studies

Author	lncRNA	Year	Country of Residence	TP	TN	FP	FN
El-Tawdi, Matboli, El-Nakeep[15]	*UCA1*	2016	Egypt	64	34	4	6
El-Tawdi, Matboli, Shehata[16]	*CTBP-AS*	2016	Egypt	71	37	7	7
Jing et al[17]	*SPRY4-IT1*	2016	China	76	32	32	11
Kamel et al[18]	*UCA1*	2016	Egypt	76	36	8	6
Kamel et al[18]	*WRAP53*	2016	Egypt	70	36	8	12
Li et al[19]	*HULC*	2015	China	45	44	9	21
Li et al[19]	*Linc00152*	2015	China	50	46	7	16
Lu et al[20]	*uc003wbd*	2015	China	110	116	22	27
Lu et al[20]	*AF085935*	2015	China	132	134	4	5
Ma et al[21]	*JPX*	2016	China	42	36	32	0
Tang et al[22]	*RP11-160H22.5, XLOC_014172,LOC149086*	2015	China	184	238	12	33
Wang et al[23]	*uc001ncr,AX800134*	2015	China	115	204	28	6
Xie et al[24]	*HULC*	2013	China	19	18	2	11
Yuan et al[25]	*Linc00152, RP11-160H22.5,XLOC_014172*	2017	China	98	96	4	2
Konishi et al[26]	*MALAT1*	2016	Japan	14	46	5	14
Zheng et al[27]	*UCA1*	2015	China	77	104	1	28
Gao, Xu et al[14]	*SHNG1*	2018	China	63	43	7	9
Sun et al[28]	*H19*	2016	China	140	195	16	40
Chao and Zhou[29]	*D16366*	2019	China	70	72	13	37
Zeng, Dong et al[30]	*X91348*	2019	China	88	62	20	19
Yang et al[31]	*HEIH*	2015	China	129	142	37	50
Zeng, Guo et al[32]	*CASC9*	2019	China	72	44	6	8
Yu et al[33]	*PVT1, uc002mbe.2*	2016	China	24	30	3	16

TP, true positive; TN, true negative; FP, false positive; FN, false negative.

Table 2. The Information on Specimen Collection and Methods of Included Studies

Author	lncRNA	Specimen	Anticoagulant	Method
El-Tawdi, Matboli, El-Nakeep[15]	*UCA1*	Serum	None	qRT-PCR
El-Tawdi, Matboli, Shehata[16]	*CTBP-AS*	Serum	None	qRT-PCR
Jing et al[17]	*SPRY4-IT1*	Plasma	EDTA	qRT-PCR
Kamel et al[18]	*UCA1*	Serum	None	qRT-PCR
Kamel et al[18]	*WRAP53*	Serum	None	qRT-PCR
Li et al[19]	*HULC*	Plasma	Nonspecified	qRT-PCR
Li et al[19]	*Linc00152*	Plasma	Nonspecified	qRT-PCR
Lu et al[20]	*uc003wbd*	Serum	None	qRT-PCR
Lu et al[20]	*AF085935*	Serum	None	qRT-PCR
Ma et al[21]	*JPX*	Plasma	EDTA	qRT-PCR
Tang et al[22]	*RP11-160H22.5, XLOC_014172,LOC149086*	Plasma	EDTA	qRT-PCR
Wang et al[23]	*uc001ncr,AX800134*	Serum	None	qRT-PCR
Xie et al[24]	*HULC*	Plasma	EDTA	qRT-PCR
Yuan et al[25]	*Linc00152, RP11-160H22.5,XLOC_014172*	Plasma	Nonspecified	qRT-PCR
Konishi et al[26]	*MALAT1*	Plasma	Nonspecified	qRT-PCR
Zheng et al[27]	*UCA1*	Serum	None	qRT-PCR
Gao, Xu et al[14]	*SHNG1*	Plasma	EDTA	qRT-PCR
Sun et al[28]	*H19*	Plasma	EDTA	qRT-PCR
Chao and Zhou[29]	*D16366*	Serum	None	qRT-PCR
Zeng, Dong et al[30]	*X91348*	Serum	None	qRT-PCR
Yang et al[31]	*HEIH*	Plasma	EDTA	qRT-PCR
Zeng, Guo et al[32]	*CASC9*	Serum	None	qRT-PCR
Yu et al[33]	*PVT1, uc002mbe.2*	Serum	None	qRT-PCR

qRT-PCR, quantitative reverse-transcription polymerase chain reaction.

Lab Medicine 2021;52;64–73 67
DOI: 10.1093/labmed/lmaa050

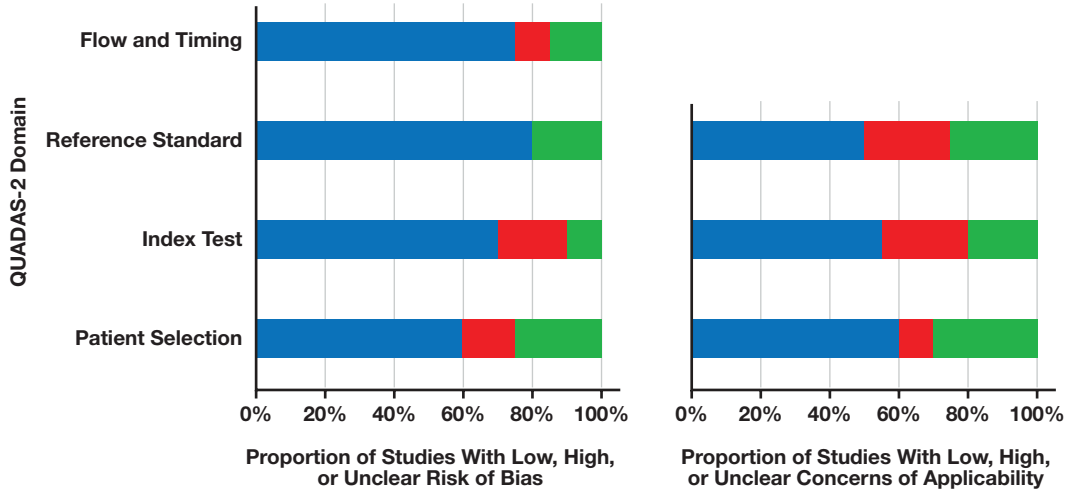


Figure 2

Risk assessment of bias and clinical applicability based on QUADAS-2.

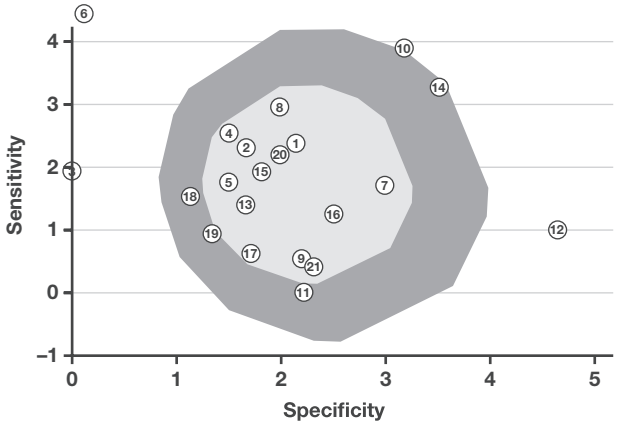


Figure 3

Bivariate boxplot for evaluating heterogeneity.

bivariate random-effects model would be an appropriate approach to conduct data synthesis.

The forest plots of the pooled sensitivity and specificity for the included studies are shown in **Figure 4**. The pooled sensitivity and specificity were 0.86 (95% confidence interval [CI], 0.80–0.90) and 0.88 (95% CI, 0.82–0.92), respectively. In addition, the pooled positive likelihood ratio (PLR), pooled negative likelihood ratio (NLR), and pooled diagnostic odds ratio (DOR) were 7.1 (95% CI, 4.9–10.2), 0.16 (95% CI, 0.11–0.23), and 44 (95% CI, 25–79), respectively. In a diagnostic test, the PLR is used to show the ratio between TP and FP results, which reveals the accuracy of a positive diagnosis. In contrast, the NLR is employed to describe the ratio between FN and TN results, which reveals the possibility of giving an incorrect negative diagnosis. Overall, given the abovementioned diagnostic performance parameters, the conclusion can be fairly drawn that lncRNAs have high diagnostic accuracy for HCC.

Threshold Effect

As shown in **Figure 5**, the summary AUC was 0.93 (95% CI, 0.91–0.95), indicating a high diagnostic performance of lncRNAs in the diagnosis of HCC. Moreover, another important function of an SROC is to observe if there is a "shoulder-arm"-shaped distribution in the SROC curve. If a shoulder-arm-shaped distribution is observed, it suggests the existence of a threshold effect that causes heterogeneity in diagnostic tests, considered to result from the differences between sensitivity and specificity. By visual inspection, we found no shoulder-arm-shaped distribution in the SROC curve. Moreover, the statistical analysis also showed that the proportion of heterogeneity because of the threshold effect was only 0.01. Therefore, we can conclude that the heterogeneity of the present meta-analysis was not associated with the threshold effect.

Publication Bias

To assess the extent of publication bias, which is a major confounding factor in reporting meta-analysis results, we used the linear regression method and the Deeks funnel plot. In **Figure 6**, each study is shown in the form of a dot.

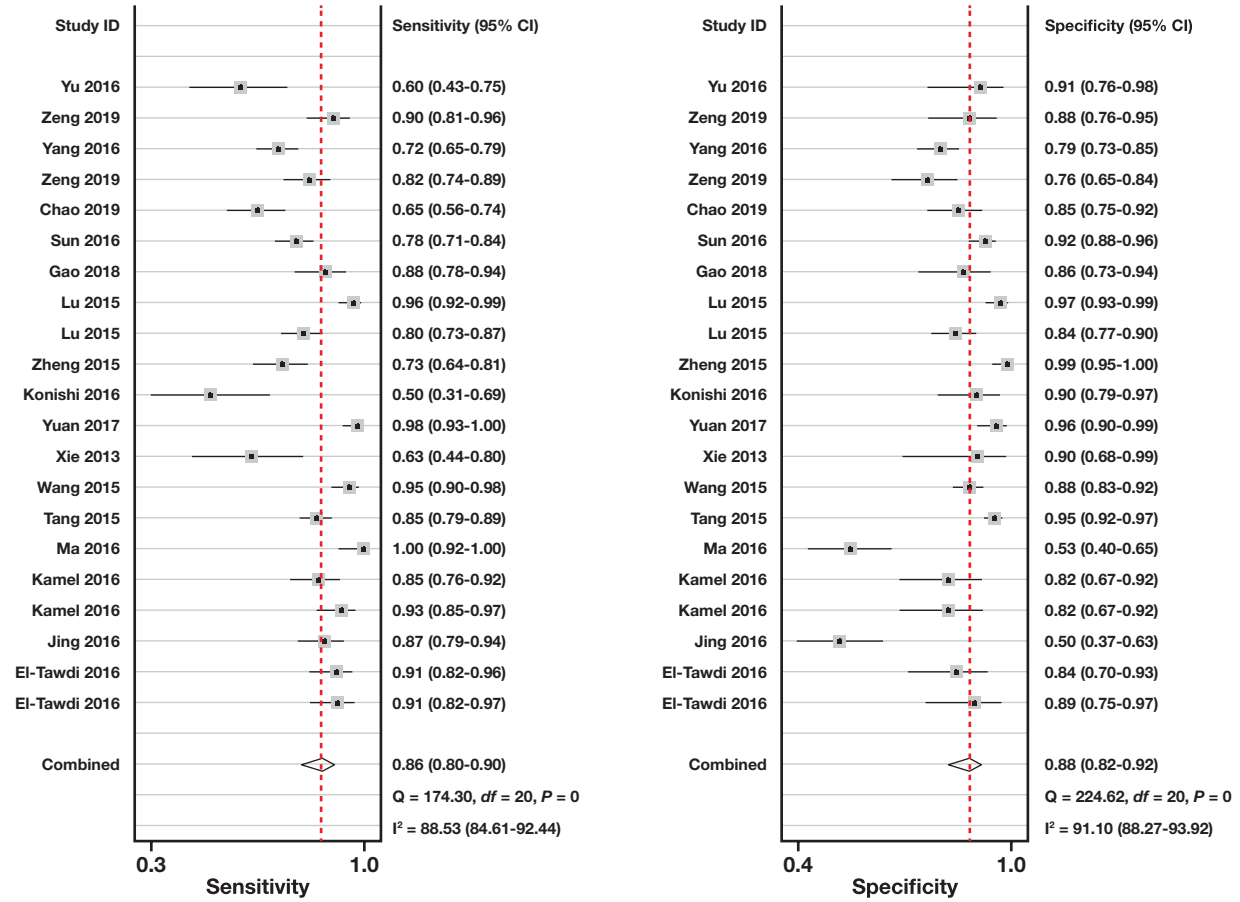


Figure 4

Forest plot of the pooled sensitivity and specificity.

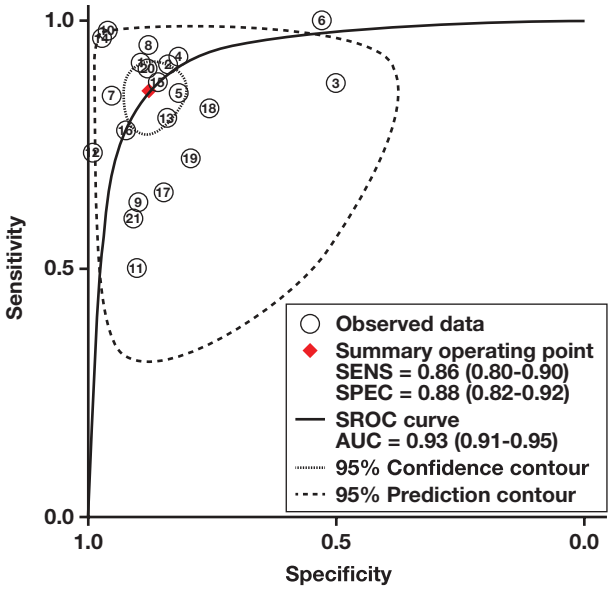


Figure 5

Summary ROC and its area under curve.

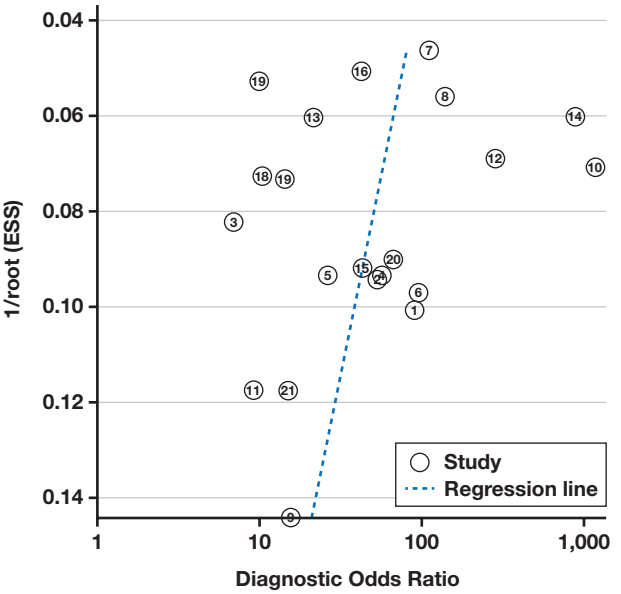


Figure 6

Deek's funnel plot for evaluating publication bias.

DOI: 10.1093/labmed/lmaa050

All studies were distributed equally on both sides of the regression line, suggesting that the publication bias was not significant. In addition, the *P* value of the linear regression method was .39, supporting the conclusion of the visual inspection.

Diagnostic Value

In the present meta-analysis, we used the Fagan nomogram to evaluate the value of lncRNAs in the diagnosis of HCC (see **Figure 7**). The pretest probability was set to 50%, which has been widely applied in previous publications, and the figure shows that the posttest probability increased to 88% if lncRNA detection was positive. The PLR of 7 suggested that a patient with HCC was 7 times more likely to obtain a positive result when compared with patient who was HCC-free. If the lncRNA result was negative, then the posttest probability would decrease to 14%. Combined with the NLR of 0.16, it is fair to conclude that lncRNAs can serve as potential biomarkers in the diagnosis of HCC.

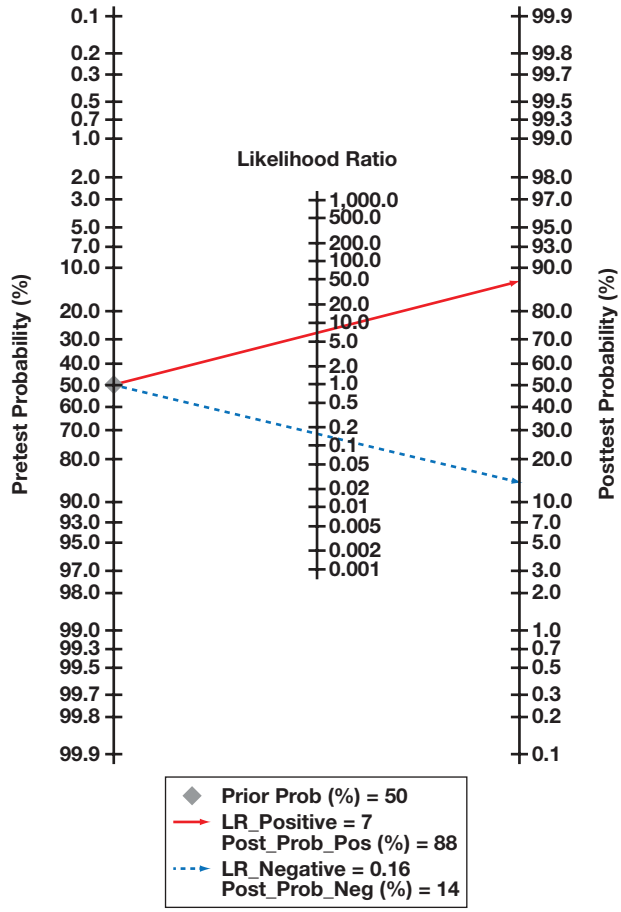


Figure 7

Fagan nomogram for evaluating diagnostic value.

Discussion

As previously described, HCC is often diagnosed at advanced stages; therefore, the prognosis is rather poor according to years of clinical observation. Currently, biomarkers that are applied in clinical practice and medical imaging approaches are not sensitive enough for the early diagnosis of HCC. As a result, clinical practice urgently needs serum biomarkers that have high sensitivity and ideal specificity to be applied in the screening and diagnosis of HCC. With the rapid development of molecular biological technology, many novel molecular biomarkers have been proven to be capable of diagnosing HCC. Zhou et al[34] employed a microarray chip to identify differentially expressed microRNAs between patients with HCC and healthy control patients; subsequently, 7 microRNAs were included in a diagnostic panel to test their diagnostic performance. The analysis results showed that this particular panel can distinguish between patients with HCC and healthy patients (AUC = 0.941) and is also capable of identifying patients with chronic HBV infection and with cirrhosis. By comparing the results of the microRNA panel and AFP, it can be concluded that this microRNA panel studied by Zhou et al[34] has superior diagnostic performance compared with AFP. Because noncoding RNAs are widely involved in carcinogenesis and the development of various kinds of cancers, lncRNAs, similar to microRNAs, have the potential to serve as serum biomarkers in diagnosing cancers.

Overall, we conducted a comprehensive literature search to identify publications reporting the diagnostic performance of lncRNAs in HCC. In total, 20 eligible studies were included in the present meta-analysis and were subjected to quality assessment and quantitative synthesis. Although significant heterogeneity was observed, the bivariate random-effects model we employed can provide a relatively robust statistical result. In addition, the SROC did not detect significant heterogeneity in the pooled sensitivities and specificities. The tests for publication bias also suggested that no significant publication bias was found. In summary, the statistical analysis of the present meta-analysis is reliable to some extent.

From the results of the quantitative synthesis, we observed a pooled sensitivity and specificity of 0.86 (95% CI, 0.80–0.90) and 0.88 (95% CI, 0.82–0.92), respectively. Moreover, the DOR reached 44 (95% CI, 25–79), indicating that a patient who had a positive result for lncRNAs would have a 39.02-fold risk of HCC when compared with a negative counterpart. The SROC curve also showed an AUC of 0.93 (95% CI, 0.91–0.95), which meant that lncRNAs had high diagnostic performance. By comparing the AUC of the present meta-analysis and historical data, it can be fairly concluded that lncRNAs have superior diagnostic performance to conventional biomarkers in HCC, such as AFP, Des-gamma-carboxy Prothrombin, and Golgi glycoprotein 73. It has been generally acknowledged that a PLR > 10 or an NLR < 0.10 is the criterion for determining whether a test can confirm or exclude a certain kind of specific disease. According to our statistical results, the PLR and NLR of our quantitative synthesis were 7.1 (95% CI, 4.9–10.2) and 0.16 (95% CI, 0.11–0.23), respectively, suggesting that lncRNAs have not yet been identified as confirmatory biomarkers. That being said, both the PLR and the NLR of lncRNAs in our study were very close to the general criteria. Therefore, we can conclude that lncRNAs have high diagnostic performance in identifying patients with HCC vs healthy control patients. In addition, we also employed the linear regression method to evaluate the publication bias of the present meta-analysis. Based on the *P* value of the test and the visual inspection of the Deeks funnel plot, the results were not significantly misled by potential publication bias.

The conception of detecting lncRNAs in the serum stems from Panzitt et al,[35] who observed a significant elevation in *HULC* in the peripheral blood specimens of patients with HCC, which was consistent with the analysis results of tissues, indicating that cancer-associated lncRNAs can be detected in blood specimens. Consequently, this finding laid a solid foundation for conducting lncRNA measurements in specimens other than tissue, including blood and other body fluids. In a genome-wide analysis of lncRNA stability performed by Clark et al,[36] the results showed that most lncRNAs have high stability and that some even have a half-life longer than 16 hours. Moreover, lncRNAs have a higher tolerance for room temperature and repeated freeze-thaw cycles, allowing them to be applied in clinical practice. With respect to the long length of lncRNAs, stem-loop primers used in detecting microRNAs are not necessary in the amplification of lncRNAs. Owing to this property, primer design can provide better specificity for lncRNAs than for microRNAs, facilitating the whole process of designing a detection method. Ren et al[37] compared the level of lncRNAs in different types of specimens and found that whereas lncRNAs in EDTA plasma and serum are more stable, heparin plasma may lead to a significant decline in lncRNA level. That being said, we only found 3 studies that did not specify the anticoagulant they used, and considering that heparin is rarely used in scientific research, we assume that the impact of specimen type may not be significant.

However, the major impediments that limit the clinical application of lncRNAs are the identification of cancer-associated lncRNAs and the consistency between lncRNA levels in tissue specimens and blood specimens. In addition, the cutoff value of an lncRNA should be ascertained by conducting large-scale population-based studies to achieve optimal diagnostic performance. Moreover, we also found that those individual studies that employed more than 1 lncRNA had satisfactory diagnostic performance[22,23,25] except the study conducted by Yu,[33] indicating that creating an lncRNA panel may increase diagnostic performance.

It has been generally acknowledged that the quality of the included literature is the core of evidence-based medicine. Having applied the QUADAS-2 tool in this research, we can conclude that the overall quality of these 20 publications is satisfactory. Nevertheless, some limitations that affect the credibility of the present meta-analysis should be addressed. First, some studies only enrolled a sample size for providing diagnostic performance parameters. Second, not all studies recruited incident patients as patients with HCC, and there were also stage differences among the enrolled patients with HCC. The abovementioned points may pose a negative impact on the conclusion drawn from our analysis. Heterogeneity among studies is another downside, and its existence may be attributed to differences in sensitivity, specificity, and sample size. Given this finding, the bivariate random-effects model is a reasonable choice.

Conclusion

To conclude, our meta-analysis suggested that lncRNAs have high diagnostic performance in HCC and therefore have the potential for clinical application. However, limited

DOI: 10.1093/labmed/lmaa050

by the small sample size of the enrolled individual publications and different lncRNAs investigated, the diagnostic performance of lncRNAs requires further investigation. **LM**

Acknowledgments

All authors have contributed to the present study. JYH, SYW and YL have equally contributed to this study. JJN conceptualized the study and revised the manuscript. JYH and SYW conducted the literature search and data entry. YL was in charge of quantitative synthesis and drafted the manuscript. HCY completed the data visualization. This work was financially supported by the Major Special Projects for Serious Illness of Xiamen (Grant No. 3502Z20179045).

References

1. Gao JD, Shao YF, Xu Y, et al. Tight association of hepatocellular carcinoma with HBV infection in North China. *Hepatobiliary Pancreat Dis Int.* 2005;4(1):46–49.
2. Petruzziello A. Epidemiology of hepatitis B virus (HBV) and hepatitis C virus (HCV) related hepatocellular carcinoma. *Open Virol J.* 2018;12:26–32.
3. Zhang X, Kang C, Li N, et al. Identification of special key genes for alcohol-related hepatocellular carcinoma through bioinformatic analysis. *PeerJ.* 2019;7:e6375.
4. Luo X, Sui J, Yang W, et al. Type 2 diabetes prevention diet and hepatocellular carcinoma risk in US men and women. *Am J Gastroenterol.* 2019;114(12):1870–1877.
5. Liu Z, Mao X, Jiang Y, et al. Changing trends in the disease burden of primary liver cancer caused by specific etiologies in China. *Cancer Med.* 2019;8(12):5787–5799.
6. Okeke E, Davwar PM, Roberts L, et al. Epidemiology of liver cancer in Africa: current and future trends. *Semin Liver Dis.* 2020;40(2):111–123.
7. Wang CY, Li S. Clinical characteristics and prognosis of 2887 patients with hepatocellular carcinoma: a single center 14 years experience from China. *Medicine (Baltimore).* 2019;98(4):e14070.
8. McIntire KR, Vogel CL, Princler GL, Patel IR. Serum alpha-fetoprotein as a biochemical marker for hepatocellular carcinoma. *Cancer Res.* 1972;32(9):1941–1946.
9. Wang Q, Chen Q, Zhang X, et al. Diagnostic value of gamma-glutamyltransferase/aspartate aminotransferase ratio, protein induced by vitamin K absence or antagonist II, and alpha-fetoprotein in hepatitis B virus-related hepatocellular carcinoma. *World J Gastroenterol.* 2019;25(36):5515–5529.
10. Lee CW, Tsai HI, Lee WC, et al. Normal alpha-fetoprotein hepatocellular carcinoma: are they really normal? *J Clin Med.* 2019;8(10):E1736.
11. Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. *Oncogene.* 2017;36(41):5661–5667.
12. Malek R, Gajula RP, Williams RD, et al. TWIST1-WDR5-hottip regulates Hoxa9 chromatin to facilitate prostate cancer metastasis. *Cancer Res.* 2017;77(12):3181–3193.
13. Hou Z, Xu X, Fu X, et al. HBx-related long non-coding RNA MALAT1 promotes cell metastasis via up-regulating LTBP3 in hepatocellular carcinoma. *Am J Cancer Res.* 2017;7(4):845–856.
14. Gao S, Xu X, Wang Y, Zhang W, Wang X. Diagnostic utility of plasma lncRNA small nucleolar RNA host gene 1 in patients with hepatocellular carcinoma. *Mol Med Rep.* 2018;18(3):3305–3313.
15. El-Tawdi AH, Matboli M, El-Nakeep S, Azazy AE, Abdel-Rahman O. Association of long noncoding RNA and c-JUN expression in hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol.* 2016;10(7):869–877.
16. El-Tawdi AH, Matboli M, Shehata HH, et al. Evaluation of circulatory RNA-based biomarker panel in hepatocellular carcinoma. *Mol Diagn Ther.* 2016;20(3):265–277.
17. Jing W, Gao S, Zhu M, et al. Potential diagnostic value of lncRNA SPRY4-IT1 in hepatocellular carcinoma. *Oncol Rep.* 2016;36(2):1085–1092.
18. Kamel MM, Matboli M, Sallam M, Montasser IF, Saad AS, El-Tawdi AHF. Investigation of long noncoding RNAs expression profile as potential serum biomarkers in patients with hepatocellular carcinoma. *Transl Res.* 2016;168:134–145.
19. Li J, Wang X, Tang J, et al. HULC and Linc00152 act as novel biomarkers in predicting diagnosis of hepatocellular carcinoma. *Cell Physiol Biochem.* 2015;37(2):687–696.
20. Lu J, Xie F, Geng L, Shen W, Sui C, Yang J. Investigation of serum lncRNA-uc003wbd and lncRNA-AF085935 expression profile in patients with hepatocellular carcinoma and HBV. *Tumour Biol.* 2015;36(5):3231–3236.
21. Ma W, Wang H, Jing W, et al. Downregulation of long non-coding RNAs JPX and XIST is associated with the prognosis of hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol.* 2017;41(2):163–170.
22. Tang J, Jiang R, Deng L, Zhang X, Wang K, Sun B. Circulation long non-coding RNAs act as biomarkers for predicting tumorigenesis and metastasis in hepatocellular carcinoma. *Oncotarget.* 2015;6(6):4505–4515.
23. Wang K, Guo WX, Li N, et al. Serum LncRNAs profiles serve as novel potential biomarkers for the diagnosis of HBV-positive hepatocellular carcinoma. *PLoS One.* 2015;10(12):e0144934.
24. Xie H, Ma H, Zhou D. Plasma HULC as a promising novel biomarker for the detection of hepatocellular carcinoma. *Biomed Res Int.* 2013;2013:136106.
25. Yuan W, Sun Y, Liu L, Zhou B, Wang S, Gu D. Circulating lncRNAs serve as diagnostic markers for hepatocellular carcinoma. *Cell Physiol Biochem.* 2017;44(1):125–132.
26. Konishi H, Ichikawa D, Yamamoto Y, et al. Plasma level of metastasis-associated lung adenocarcinoma transcript 1 is associated with liver damage and predicts development of hepatocellular carcinoma. *Cancer Sci.* 2016;107(2):149–154.
27. Zheng ZK, Pang C, Yang Y, Duan Q, Zhang J, Liu WC. Serum long noncoding RNA urothelial carcinoma-associated 1: a novel biomarker for diagnosis and prognosis of hepatocellular carcinoma. *J Int Med Res.* 2018;46(1):348–356.
28. Sun YK, Zhao F, Wang F, et al. Diagnostic value of long non-coding RNA H19 in primary hepatic carcinoma. *Chin J Health Lab Tec*. 2016; 26(3):305–310.
29. Chao Y, Zhou D. lncRNA-D16366 is a potential biomarker for diagnosis and prognosis of hepatocellular carcinoma. *Med Sci Monit.* 2019;25:6581–6586.
30. Zeng Z, Dong J, Li Y, et al. The expression level and clinical significance of lncRNA X91348 in hepatocellular carcinoma. *Artif Cells Nanomed Biotechnol.* 2019;47(1):3067–3071.
31. Yang Z, Li J, Ma Z, et al. Association between expression of lncRNA-HEIH in plasma and hepatocellular carcinoma risk: a case-control study. *Mod Oncol.* 2015; 23(1):80–84.

32. Zeng YL, Guo ZY, Su HZ, Zhong FD, Jiang KQ, Yuan GD. Diagnostic and prognostic value of lncRNA cancer susceptibility candidate 9 in hepatocellular carcinoma. *World J Gastroenterol.* 2019;25(48):6902–6915.

33. Yu J, Han J, Zhang J, et al. The long noncoding RNAs PVT1 and uc002mbe.2 in sera provide a new supplementary method for hepatocellular carcinoma diagnosis. *Medicine (Baltimore).* 2016;95(31):e4436.

34. Zhou J, Yu L, Gao X, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol.* 2011;29(36):4781–4788.

35. Panzitt K, Tschernatsch MM, Guelly C, et al. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. *Gastroenterology.* 2007;132(1):330–342.

36. Clark MB, Johnston RL, Inostroza-Ponta M, et al. Genome-wide analysis of long noncoding RNA stability. *Genome Res.* 2012;22(5):885–898.

37. Ren S, Wang F, Shen J, et al. Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 derived miniRNA as a novel plasma-based biomarker for diagnosing prostate cancer. *Eur J Cancer.* 2013;49(13):2949–2959.

DOI: 10.1093/labmed/lmaa050

Transfusion Requirements and Blood Bank Support in Heart and Lung Transplantation

Dong-Won Yoo, MD,[1] Hyun-Ji Lee, MD, PhD,[1] Seung-Hwan Oh, MD, PhD,[1] In Suk Kim, MD, PhD,[1] Hyung-Hoi Kim, MD, PhD,[2] Hyung Gon Je, MD, PhD,[3] Dohyung Kim, MD,[3] Woo Hyun Cho, MD, PhD,[4] Jeong Su Kim, MD, PhD,[5] Soo Yong Lee, MD, PhD,[5] Hye Ju Yeo, MD, PhD[4]*

Laboratory Medicine 2021;52:74-79

DOI: 10.1093/labmed/lmaa044

ABSTRACT

Objective: Transplantations may require massive transfusion of blood products. Therefore, blood banks need to predict, prepare, and supply the required amount of blood products.

Methods: We measured the volume of transfused blood components as red blood cells, fresh frozen plasma, platelets, and cryoprecipitate in 54 and 89 patients who received heart and lung transplantation, respectively, in our hospital between January 2012 and December 2019.

Results: Platelets were the most frequently transfused blood component. Transfusion volumes during heart and lung transplantation surgeries differed: red blood cells, 7.83 units vs 14.84 units; fresh frozen plasma, 2.67 units vs 12.29 units; platelets, 13.13 units vs 23.63 units; and cryoprecipitate, 1.74 units vs 2.57 units; respectively. The average transfusion volume of transplants was different each year.

Conclusion: Periodic evaluation of transfusion requirements will facilitate the efficient management of blood products at the time of transplantation and help blood banks predict changes in blood requirements.

Keywords: solid organ transplantation, transfusion, intraoperative, surgical blood ordering schedule, massive blood transfusion, blood bank

First described in 1976, the maximum surgical blood ordering schedule (MSBOS) was created to provide guidelines and recommendations for preoperative pretransfusion testing and ordering of red blood cell (RBC) units.[1] The MSBOS is a table of elective surgical procedures that lists the RBC units that are routinely cross-matched preoperatively for each procedure.[2] The MSBOS was introduced in Korea in the 1980s for use as a guide for blood transfusions during surgery. However, guidelines for transfusion volume may vary among different medical institutions. A hospital may create its own standard guidelines by investigating the amount of blood transfused for surgery, calculating statistics, and setting its own MSBOS.

Because of improved physician skills and surgical techniques along with changes in perceptions about transfusion, the amount of transfusion products required for the same surgery may change over time.[3] It is necessary to study the transfusion data of individual hospitals to prepare a stable supply of blood products. Organ transplant surgery is known to require a high volume of blood transfusions,[4] but only a few studies have reported on transfusions or the MSBOS of transplants.[3-5]

In this study, we investigated blood transfusion volumes that were transfused preoperatively, intraoperatively, and

Abbreviations:

MSBOS, maximum surgical blood ordering schedule; RBC, red blood cell; HT, heart transplantation; LT, lung transplantation; FFP, fresh frozen plasma; PLT, platelet concentrates; ECMO, extracorporeal membrane oxygenation; RIS, rapid infusion system; Surgery can result in excessive bleeding, often because of unexpected conditions; therefore, surgeons generally order sufficient supplies for blood transfusion in advance. However, because of the limited availability of blood in blood banks, blood bank managers strive to optimize the use of blood products.

[1]Department of Laboratory Medicine and Biomedical Research Institute, Pusan National University Yangsan Hospital, Yangsan, Korea, [2]Department of Laboratory Medicine, Pusan National University Hospital, Pusan, Korea, [3]Department of Cardiovascular and Thoracic Surgery, Research Institute for Convergence of Biomedical Science and Technology and [4]Department of Pulmonology and Critical Care Medicine, Pusan National University Yangsan Hospital, Yangsan, Korea, [5]Division of Cardiology, Department of Internal Medicine and Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Pusan National University School of Medicine, Yangsan, Korea

*To whom correspondence should be addressed.
hilhj1120@gmail.com

postoperatively in heart and lung transplants. This study was conducted to evaluate the changes in transfusion requirements and predict transfusion patterns during transplantation of both these organs.

Materials and Methods

Study Design

We retrospectively included 54 patients who received heart transplantation (HT) and 89 patients who received lung transplantation (LT) in our tertiary hospital from January 2012 to December 2019. We measured the amount of blood products transfused preoperatively (from hospitalization to surgery), intraoperatively (during surgery), and postoperatively (from 1 day after surgery to the discharge date). Blood products included red blood cells (RBCs), fresh frozen plasma (FFP), platelet concentrates (PLT), apheresis platelets, and whole blood–derived cryoprecipitate (cryo). One unit of apheresis platelets was calculated retrospectively as being equivalent to 6 units of whole blood–derived PLT. Therefore, the number of platelet units was counted as both single whole blood–derived units and calculated units from apheresis platelets. The packed RBC blood products included both leukoreduced and irradiated RBCs. Irradiated blood components are treated with radiation to prevent transfusion-associated graft-versus-host disease.

Data Acquisition

Patient information was obtained, and data from the last complete blood count and liver renal function test were recorded. All laboratory test results were preoperatively measured, and the time of measurement was the last result reported on the day of the surgery. Age, sex, and laboratory results, including hemoglobin, platelet, total bilirubin, albumin, creatinine, prothrombin time, and activated partial thromboplastin time, were investigated. In addition, patients undergoing high-risk surgical procedures requiring extracorporeal membrane oxygenation (ECMO) were included.

Each patient's diagnosis was ascertained through a review of the hospital's electronic medical records. This study was approved by the institutional review board of our hospital after a full committee review (approval no. 05-2018-066).

Data Extraction and Analysis

The age, sex, and preoperative blood test results for each group were obtained by calculating the mean and standard deviation or ratio. The results of each patient group for HT and LT were analyzed statistically, and the amounts of blood products transfused pre-, intra-, and post-transplantation were investigated according to the transplanted organs. The blood transfusions were analyzed statistically by the type of blood product, such as RBC, platelets, FFP, and cryo. Statistical significance of the results was calculated by using nonparametric tests: the Mann-Whitney *U*-test for continuous variables and Fisher exact test and paired *t*-test for categorical variables. Statistical tests were conducted using SPSS version 22.0 software (SPSS, Inc, Chicago, IL). Results were considered statistically significant for $P < .05$.

Results

The background characteristics of the 2 patient groups are shown in **Table 1**. Fifty-four patients received HT and 89 patients received LT in our hospital. Among the patients undergoing LT, 52 patients received a single-lobe transplant and 37 patients received a double-lobe transplant. The frequency of ECMO was much higher in patients who received LT than in patients who received HT. In the HT group, 27 patients were diagnosed with dilated cardiomyopathy, 14 patients had congestive heart failure, and 11 patients were diagnosed with heart failure because of acute myocarditis. The commonest disease among patients who received LT was idiopathic pulmonary fibrosis (40 patients), followed by acute respiratory distress syndrome (19 patients) and interstitial lung disease (9 patients). Other patients (1 each for each condition) in the LT group were diagnosed with pneumococcal pneumonia, chronic obstructive pulmonary disease, chronic respiratory failure, acute respiratory failure, and bronchiolitis obliterans with organizing pneumonia.

The analysis of preoperative, intraoperative, and postoperative transfusion volumes in patients who received HT and LT is shown in **Figure 1** and **Table 2**. The most frequently transfused blood product was platelets, followed by RBCs, FFP, and cryo. In HT and LT, platelets were the most intraoperatively transfused blood product, and the intraoperative transfusion volume was higher than the preoperative transfusion volume.

Lab Medicine 2021;52:74–79
DOI: 10.1093/labmed/lmaa044

Table 1. Demographic Data of Study Participants

Characteristics	Heart (n = 54)	Lung (n = 89)
Mean age, y (SD)	43.98 (16.13)	45.76 (12.13)
Male (%)	35 (63)	60 (67.4)
Female (%)	19 (37)	29 (32.6)
Hb, g/dL (SD)	10.69 (2.0)	10.63 (2.1)
PLT, $10^3/\mu L$ (SD)	172.78 (78.07)	167.10 (77.74)
Total bilirubin, mg/dL (SD)	2.15 (2.16)	1.05 (0.76)
Albumin, g/DL (SD)	3.49 (0.59)	3.05 (0.69)
Creatinine, mg/dL (SD)	1.00 (0.51)	0.68 (0.54)
PT, sec (SD)	16.74 (4.43)	14.49 (2.71)
aPTT, sec (SD)	58.54 (37.51)	46.84 (13.55)
ECMO (%)	19 (35.2)	80 (89.9)
Diagnosis		
DCMP	27	…
Congestive heart failure	14	…
Heart failure due to acute myocarditis	11	…
Hypertrophic cardiomyopathy	2	…
IPF	…	40
Interstitial lung disease	…	9
Influenza ARDS	…	19
Primary pulmonary hypertension	…	4
TB-related lung disease	…	4
Bronchiolitis obliterans	…	5
Others	…	8

DCMP, dilated cardiomyopathy; IPF, idiopathic pulmonary fibrosis; ARDS, acute respiratory distress syndrome; TB, tuberculosis; SD, standard deviation; PT, prothrombin time; aPTT, activated partial thromboplastin time; Hb, hemoglobin, ECMO, extracorporeal membrane oxygenation.
Other diagnoses included pneumocystis pneumonia (n = 1), chronic obstructive pulmonary disease (n = 1), chronic respiratory failure (n = 1), acute respiratory failure (n = 1), and bronchiolitis obliterans with organizing pneumonia (n = 1).

In patients who received LT, the amount of transfused cryo was not significantly different preoperatively and postoperatively (*P* value =.055). There was no statistically significant difference between the intraoperative and postoperative transfusion volume of RBC, FFP, and cryo blood products (*P* value =.17, .104, and.794, respectively).

The blood transfusion volume was investigated every year, and the transfusion volume relative to RBC was calculated (**Table 3**). Since 2016, the average number of transplant operations increased to more than 10 on average (HT = 12.5, LT = 18.5 in 2016–2019). In HT, the transfusion volume of FFP and cryo was relatively less than that of RBC during the entire study period. However, platelet transfusion was relatively higher in the last 4 years of the study than RBC transfusion. The average ratio of RBC:FFP:platelets:cryo over the past 4 years (2016–2019) was 1.00:0.36:1.86:0.19 and 1.00:0.92:1.94:0.04 in the HT and LT groups, respectively. The results showed that the relative proportion of blood transfusions varies by transplant organ.

Discussion

Appropriate transfusion support is needed to increase the success rate of HT and LT. To support transfusion, it is

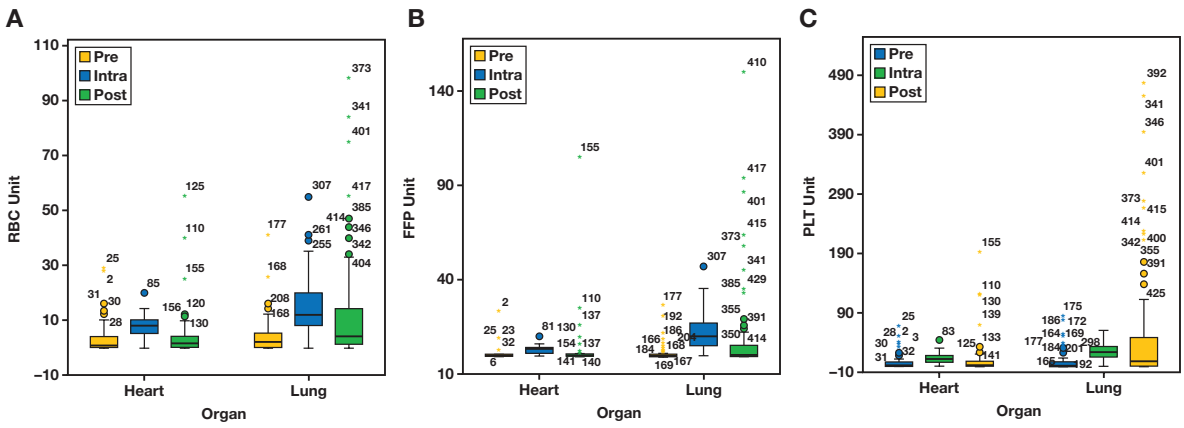


Figure 1

Transfusion requirements for RBCs **(A)**, FFP **(B)**, and PLT **(C)** in the pretransplantation, intraoperative, and posttransplantation phase in HT and LT. RBC, red blood cell; FFP, fresh frozen plasma; PLT, platelet concentrations; HT, heart transplantation; LT, lung transplantation.

Table 2. Comparison of Mean and Range of Units of Blood Products for Transfusion and Number of Blood Products by HT and LT

Organ	Category	RBC (units)	FFP (units)	PLT (units)	Cryo (units)
Heart (n = 54)	Preoperative	3.44 (0–29)	0.91 (0–24)	6.39 (0–67)	0.33 (0–18)
	P^a value	<.001	.005	.001	.005
	Intraoperative	7.83 (0–20)	2.67 (0–10)	13.13 (0–44)	1.74 (0–20)
	P^b value	.018	.821	.997	.008
	Postoperative	4.46 (0–55)	3.13 (0–105)	13.11 (0–192)	0.35 (0–10)
	P^c value	0.464	0.269	0.144	0.931
Lung (n = 89)	Preoperative	3.90 (0–41)	1.48 (0–26)	10.20 (0–84)	0.57 (0–20)
	P^a value	<.001	<.001	<.001	.007
	Intraoperative	14.84 (0–55)	12.29 (0–47)	23.63 (0–60)	2.57 (0–30)
	P^b value	.17	.104	.004	.794
	Postoperative	11.98 (0–98)	8.30 (0–150)	54.61 (0–477)	2.88 (0–88)
	P^c value	<.001	.007	<.001	.055

HT, heart transplantation; LT, lung transplantation; RBC, red blood cells; FFP, fresh frozen plasma; PLT, platelet concentrations; cryo, cryoprecipitate.
[a]P value, paired t-test between preoperative and intraoperative groups.
[b]P value, paired t-test between intraoperative and postoperative groups.
[c]P value, paired t-test between preoperative and postoperative groups.

Table 3. Average Units of Intraoperatively Transfused Blood Products and Ratio to RBC per Year

Year	Heart (unit)					Lung (unit)				
	n	RBC (ratio)	FFP (ratio)	PLT (ratio)	Cryo (ratio)	n	RBC (ratio)	FFP (ratio)	PLT (ratio)	Cryo (ratio)
2012	…	…	…	…	…	4	27.8 (1.00)	19.8 (0.71)	20.0 (0.72)	1.8 (0.06)
2013	…	…	…	…	…	2	19.5 (1.00)	15.0 (0.77)	16.0 (0.82)	23.0 (1.18)
2014	2	10.5 (1.00)	0.0 (0.00)	12.0 (1.14)	7.5 (0.71)	4	26.8 (1.00)	11.3 (0.42)	34.5 (1.29)	18.8 (0.70)
2015	2	8.5 (1.00)	1.0 (0.12)	8.0 (0.94)	0.0 (0.00)	5	20.8 (1.00)	13.8 (0.66)	44.0 (2.12)	15.0 (0.72)
2016	10	8.4 (1.00)	2.8 (0.33)	18.7 (2.23)	1.4 (0.17)	12	11.2 (1.00)	10.3 (0.92)	22.7 (2.03)	0.0 (0.00)
2017	8	8.1 (1.00)	2.3 (0.28)	12.3 (1.51)	1.3 (0.15)	14	6.6 (1.00)	6.2 (0.94)	13.9 (2.09)	0.0 (0.00)
2018	12	11.5 (1.00)	4.1 (0.35)	20.4 (1.78)	3.8 (0.33)	11	13.6 (1.00)	12.6 (0.93)	25.8 (1.89)	2.4 (0.17)
2019	20	4.9 (1.00)	2.4 (0.48)	9.6 (1.95)	0.5 (0.10)	37	15.8 (1.00)	14.1 (0.89)	27.3 (1.73)	0.0 (0.00)

RBC, red blood cells; FFP, fresh frozen plasma; PLT, platelet concentrations; cryo, cryoprecipitate.
N is the number of transplantation surgeries.

important to accurately estimate the amount of transfusion products needed before and during transplantation. For example, in organ transplantation, the lungs and heart can be donated from a living donor or one who is brain-dead. Unlike with organ transplantation from living donors, brain death is always unexpected and thus there is less time to arrange blood replacement supplies than with an elective surgery.[6] Another consideration is plasma replacement: before transplantation, recipients in our study with donor-specific human leukocyte antigen antibodies underwent therapeutic plasma exchange.[7] The depleted plasma should be replenished by a replacement fluid, such as 4% to 5% albumin solution or FFP. In addition, some patients in our study used ECMO to support the role of the heart and lungs before organ transplantation, and this led to a change in blood transfusion requirements.[8-10] Studies of blood transfusion can be used to estimate the amount of blood products needed or to analyze optimal volumes of blood replacement.[11-14] Our study investigated the amount of blood transfusion products used in HT and LT at a single center from the beginning of its HT and LT program for a number of years.

Furthermore, this study examined the results of previous regional and international studies on blood transfusion in transplants. In 1997, 3 patients at a tertiary hospital in Korea underwent HT. The mean transfusion requirements were RBC, 2.6 units; FFP, 5.6 units; PLT, 7.3 units; and apheresis platelets, 0.3 units.[11] Two studies on LT in other Korean medical institutions reported an average RBC transfusion of

DOI: 10.1093/labmed/lmaa044

11 units.[4] One study conducted 2192 heart transplantations for 1 year and transfused on average 2 to 4 units of RBC, 1.6 units of FFP, and 1 unit of platelets during surgery.[12] The average number of blood products used for 514 lung transplants at a university hospital in the Netherlands were RBC, 4 units; FFP, 2 units; and platelets, zero units.[13] In another international study, 311 patients who underwent bilateral LT were evaluated for transfusion requirement, and the mean transfusion requirement was RBC, 3 units; FFP, 2 units; and platelets, 1 unit.[14]

Comparison of blood transfusion volumes in the existing regional and international studies showed that our average blood transfusion volume was higher in both HT and LT. Regardless of the type of blood product, all blood products were transfused in higher amounts than in the transfusion volumes in previously reported studies. A number of factors affect the amount of transfusion during transplantation, including surgeon proficiency and skill and surgical equipment. The rapid infusion system (RIS) is a device that rapidly transfuses blood at a constant temperature. The reservoir and circuitry must be filled with more than 700 mL of blood at all times. If a large amount of blood is needed, such as in the case of a transplant, a large reservoir (300 mL) is prepared and filled with higher volumes of blood components.[15] In our tertiary care hospital, the use of RIS for HT and LT is expected to increase the volume of transfusions.

The experience of the transplant surgeon determines the transfusion volume. For instance, when LT was first conducted at a tertiary care hospital in Korea, an average of 32.1 units of RBC were transfused per operation.[16] However, after 300 patients there received transplantation, the transfusion requirement for successful transplantation decreased to an average of only 5.5 units of RBC. This previous study reported that surgical technique and experience are the most important factors that influence blood transfusion volume. However, advances in surgical experience or technology may not be the only criteria for predicting the volume of blood transfusions.

In our study, PLT was transfused more than other blood products. Research has shown that ECMO can cause an increase in platelet transfusions. In a study of adult patients treated using ECMO, 5 units of RBCs and 9 units of platelets were transfused per day.[10] Another study reported that 41 adult patients who underwent ECMO received an average of 2 units of RBC and 3 units of platelets per day.[8] In the Butch et al study,[9] the daily transfusion units of packed RBC, PLT, and FFP in patients treated using ECMO were 3.2 units, 13.6 units, and 0.1 units, respectively. In their study, the largest transfusion agent in patients treated using ECMO was PLT, accounting for 80% of the total blood products. This amount is similar to the blood transfusion distribution shown in our study.

The transfusion volume depends on the patient's disease and on preoperative and postoperative conditions, including age, risk of preoperative bleeding, and underlying disease. Even for the same operation, the blood transfusion volume varies from hospital to hospital.[17] For example, hospitals with a higher percentage of high-risk patients may have a higher blood transfusion requirement than other hospitals.[18] In this study, the intraoperative transfusion volume of RBCs in patients who received LT continued to decrease over several years, but afterward it showed an increase that was probably attributable to the increased proportion of high-risk patients with severe pulmonary disease. Therefore, it is necessary to analyze patients' risk factors and statistical analysis to predict transfusion volume.

Limitations

This study investigated the amount of blood transfusion needed for HT and LT. However, when compared with the results of international studies, an insufficient number of patients who received organ transplant was a limitation of this work. If there is a statistical analysis result that can predict the volume of blood transfusion needed in a given time period, then it is possible for clinicians to intervene to control it. There was no intervention on our part to change the blood product utilization during the study period. It is difficult to generalize the amount of blood products needed because of the change in average transfusion volume every year. Therefore, this statistical analysis did not change the blood-ordering practices for transfusion. This study examined the results of laboratory diagnostic tests before transplants; postoperative laboratory results were not undertaken and could have predicted the volume of posttransplant blood transfusions. There was a period of increased transfusion volume. We assumed that was related to the severity of patient risk, but it was also a limitation that the risk analysis was not correctly performed.

Conclusion

Massive transfusion is defined as the transfusion of 8 to 10 units of packed RBCs within 24 hours.[19] Each HT and LT requires transfusion volumes that are comparable to that of a massive transfusion. In hospitals that prepare for transplants, a sufficient number of blood components are needed, and it is also important for blood banks to maintain adequate blood replacement supplies. A lack of blood products could delay needed transfusions, and stocking excessive quantities of blood products can increase wastage. Therefore, for efficient management of blood resources, periodic blood transfusion evaluation will facilitate efficient preparedness for the transfusion of blood products during transplantations. **LM**

Acknowledgments

This study was supported by the Research Institute for Convergence of Biomedical Science and Technology (30-2017-020), Pusan National University Yangsan Hospital, Yangsan, Korea. The researchers claim no conflicts of interest.

References

1. Woodrum CL, Wisniewski M, Triulzi DJ, Waters JH, Alarcon LH, Yazer MH. The effects of a data driven maximum surgical blood ordering schedule on preoperative blood ordering practices. *Hematology.* 2017;22(9):571–577.
2. Friedman BA, Oberman HA, Chadwick AR, Kingdon KI. The maximum surgical blood order schedule and surgical blood use in the United States. *Transfusion.* 1976;16(4):380–387.
3. Makroo R, Raina V, Gupta S, et al. Maximum surgical blood order schedule (MSBOS) in liver transplant. *Apollo Medicine.* 2008;5(4):383–386.
4. Park JR, Alghamdi E, Kim S, Kim HO. An update of maximum surgical blood order schedule in elective surgery. *Korean J Blood Transfus.* 2015;26(1):38–46.
5. La Jeon Y, Lee W-I, Kang SY, et al. Establishment of maximum surgical blood order schedule (MSBOS) and evaluation of effective blood usage in major surgery. *Korean J Blood Transfus.* 2018;29(1):41–50.
6. Mascia L, Mastromauro I, Viberti S, Vincenzi M, Zanello M. Management to optimize organ procurement in brain dead donors. *Minerva Anestesiol.* 2009;75(3):125–133.
7. Jackups R Jr, Canter C, Sweet SC, Mohanakumar T, Morris GP. Measurement of donor-specific HLA antibodies following plasma exchange therapy predicts clinical outcome in pediatric heart and lung transplant recipients with antibody-mediated rejection. *J Clin Apher.* 2013;28(4):301–308.
8. Ang AL, Teo D, Lim CH, Leou KK, Tien SL, Koh MB. Blood transfusion requirements and independent predictors of increased transfusion requirements among adult patients on extracorporeal membrane oxygenation—a single centre experience. *Vox Sang.* 2009;96(1):34–43.
9. Butch SH, Knafl P, Oberman HA, Bartlett RH. Blood utilization in adult patients undergoing extracorporeal membrane oxygenated therapy. *Transfusion.* 1996;36(1):61–63.
10. Omar HR, Mirsaeidi M, Socias S, et al. Plasma free hemoglobin is an independent predictor of mortality among patients on extracorporeal membrane oxygenation support. *PLoS One.* 2015;10(4):e0124034.
11. Jeong GY, Choi JS, Kim DW, Kim GS, Joh JW, Lee SK. Optimal blood ordering for solid organ transplantation. *Korean J Blood Transfus.* 1997;8(2):157.
12. Sarkar RS, Philip J, Yadav P. Transfusion medicine and solid organ transplant—update and review of some current issues. *Med J Armed Forces India.* 2013;69(2):162–167.
13. Cernak V, Oude Lansink-Hartgring A, van den Heuvel ER, et al. Incidence of massive transfusion and overall transfusion requirements during lung transplantation over a 25-year period. *J Cardiothorac Vasc Anesth.* 2019;33(9):2478–2486.
14. Ong LP, Thompson E, Sachdeva A, et al. Allogeneic blood transfusion in bilateral lung transplantation: impact on early function and mortality. *Eur J Cardiothorac Surg.* 2016;49(2):668–674.
15. Hong CK, Song HG. Clinical experience with using a rapid infuse at an urban emergency department. *J Korean Soc Traumatol.* 2006;19(1):41.
16. Song JG, Kim HJ, Gwak MS, Choi SJ, Kim GS, Lee JU. Assessment of progressive changes in the amount of blood transfusion of adult liver transplantation. *Korean J Anesthesiol.* 2005;48(1):33.
17. Karkouti K, Wijeysundera DN, Beattie WS, et al.; Reducing Bleeding in Cardiac Surgery (RBC) Research Group. Variability and predictability of large-volume red blood cell transfusion in cardiac surgery: a multicenter study. *Transfusion.* 2007;47(11):2081–2088.
18. Ejaz A, Spolverato G, Kim Y, Frank SM, Pawlik TM. Variation in triggers and use of perioperative blood transfusion in major gastrointestinal surgery. *Br J Surg.* 2014;101(11):1424–1433.
19. Green L, Tan J, Grist C, Kaur M, MacCallum P. Aetiology and outcome of massive transfusion in two large London teaching hospitals over a 3-year period (2012–2014). *Transfus Med.* 2017;27(5):342–347.

Clinical Value of Detecting Anti-Mutated Citrullinated Vimentin, Anti-Cyclic Citrullinated Peptide, Red Cell Distribution Width and 25-Hydroxyvitamin D in the Diagnosis of Rheumatoid Arthritis

Liming Tan, BS Med,[1] Yangyang Gong, BS Med,[2] Qian Zhang, BS Med,[2] Haocheng Zhang, BS Med,[2] Xiaoxia Lu, BS Med,[2] Huijin Huang, BS Med[3]*

Laboratory Medicine 2021;52:80-85

DOI: 10.1093/labmed/lmaa040

ABSTRACT

Objectives: To investigate the clinical value of detecting anti–mutated citrullinated vimentin (anti-MCV), anti-citrullinated peptide (anti-CCP), red-blood-cell distribution width (RDW), and 25-hydroxyvitamin D (25-[OH]D) in the diagnosis of rheumatoid arthritis (RA).

Methods: We enrolled 119 patients with RA, 114 control individuals without RA (*disease controls*), and 40 healthy controls in our study (Han Chinese). Anti-CCP and anti-MCV were detected by enzyme-linked immunosorbent assay (ELISA), 25-(OH)D was detected by electrochemical luminescence, and RDW was calculated by erythrocyte parameters detected via the electric resistance method.

Results: The serum levels of anti-CCP and anti-MCV in RA were higher than those in disease controls and healthy controls (P <.01). The areas under the curve (AUCs) of anti-MCV, anti-CCP, RDW, and 25-(OH)D were 0.857, 0.890, 0.611, and 0.569 respectively (P <.05). In various combinations of indicators, when RDW, 25-(OH)D, and anti-CCP; or RDW, 25-(OH)D, anti-CCP, and anti-MCV were connected in parallel, the sensitivity was the highest (all 94.1%). Also, when RDW, 25-(OH)D, anti-CCP, and anti-MCV were connected in series, the sensitivity was the lowest (13.4%).

Conclusions: Anti-CCP and anti-MCV are ideal indices for RA diagnosis. Also, in combination with RDW and 25-(OH)D, the diagnostic level will be improved, as well as the sensitivity and specificity, which is significant for the differential diagnosis of RA.

Keywords: rheumatoid arthritis, anti-mutated citrullinated vimentin, anti–cyclic citrullinated peptide, 25-hydroxyvitamin D, red-blood-cell distribution width, diagnosis

Abbreviations:

RA, rheumatoid arthritis; anti-CCP, anti–cyclic citrullinated peptide; anti-MCV, anti–mutated citrullinated vimentin; RDW, red blood cell distribution width; 25-(OH)D, 25-hydroxyvitamin D; SLE, systemic lupus erythematosus; SS, Sjögren syndrome; AS, ankylosing spondylitis; ELISA, enzyme-linked immunosorbent assay; K-s, Kolmogorov-Smirnov; ANOVA, analysis of variance; ROC, receiver operating characteristic; PPV, positive predictive value; NPV, negative predictive value; RF, rheumatoid factor; ACR/EULAR, American College of Rheumatology/European League Against Rheumatism; AUC, area under the curve; CI, confidence interval; Sens, sensitivity; Spec, specificity; YI, Youden index; +LR, positive likelihood ratio; –LR, negative likelihood ratio; … , nonapplicable

[1]Department of Clinical Laboratory, the Second Affiliated Hospital of Nanchang University, Jiangxi Province Key Laboratory of Laboratory Medicine and [2]School of Public Health, Nanchang University, Nanchang, China, [3]Jiangxi Province JiuJiang Maternal and Child Health Care Center, JiuJiang, China

*To whom correspondence should be addressed.
ndefy84029@ncu.edu.cn

Rheumatoid arthritis (RA) is a systemic autoimmune disease that is mainly related to strain on the joints. Its pathogenesis is unclear, which has a great impact on the life and work of affected patients.[1] The clinical diagnosis of RA is mainly based on X-ray examination, serological examination, and clinical performance. Clinical practice has confirmed that the diagnostic accuracy of clinical diagnosis is low; hence, misdiagnosis and missed diagnosis easily occur. Therefore, it is particularly important to find the diagnostic index with high diagnostic value or a joint test of the existing index.

It has been reported in the literature[2,3] that anti-cyclic citrullinated peptide (anti-CCP) is a serum marker with high sensitivity and specificity, and has high clinical diagnostic value. Anti–mutated citrullinated vimentin (anti-MCV) is one of the hot spots in RA research in recent years.[4] Red blood

cell distribution width (RDW) is usually used as a related indicator of anemia, and the study of RDW in RA has gradually increased recently.[5] Results from the further study of 25-hydroxyvitamin D (25-[OH]D) have shown that this analyte has an immunological regulation effect. Specifically, study results[6–8] have shown that 25-(OH)D levels in patients with RA were generally reduced and related to disease activity.

Between January 2017 and July 2019, we recruited 119 patients with RA, 114 patients without RA, and 40 healthy individuals of Han ethnicity at the Second Affiliated Hospital of Nanchang University, Nanching, China for our study. Our aim was to explore the clinical significance of detecting anti-MCV, anti-CCP, RDW, and 25-(OH)D in the diagnosis of RA.

Materials and Methods

Subject Individuals

All subjects were confirmed outpatients or inpatients in the Second Affiliated Hospital of Nanchang University from January 2017 through July 2019. The 119 patients with RA ranged in age from 23 years through 83 years. The disease state of all patients conformed to the 2010 American College of Rheumatology revised diagnosed criteria for RA.[9] In total, 114 patients without RA, who had been admitted to the Second Affiliated Hospital of Nanchang University during the same period, were selected as the disease controls, including 41 patients with of systemic lupus erythematosus (SLE; ages range, 20 years–74 years), 27 patients with Sjögren syndrome (SS; 31–75 years); 30 patients with arthritis (29–71 years); and 16 patients with ankylosing spondylitis (AS; 34–61 years). The 40 healthy controls (aged 22–80 years) had all undergone physical examination in the physical examination center of the hospital. The diagnosis of all patients was confirmed by 2 specialist physicians, and all specimens were obtained with the consent of the patients. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University.

Inclusion Criteria

The inclusion criteria were as follows. First, all participants had signed informed consent and volunteered to participate in the study. Second, the diagnosis of each patient participant was clear, and the clinical data were complete. Third, patients with major diseases (such as malignant cancer, cerebrovascular disease, and acute myocardial infarction) were excluded by reassessment.

Exclusion Criteria

The exclusion criteria were as follows. First, patients suffered from serious diseases of the liver, brain, heart, kidney, and circulatory system. Second, patients harbored acute and chronic infectious diseases, such as septicemia, traumatic infection, and bacterial infection. Third, patients had diseases that affect the absorption and metabolism of vitamin D. Fourth, patients had other autoimmune diseases, osteoporosis, or severe anemia.

Instruments, Reagents, and Their Uses

Two specimens of fasting venous blood were drawn from all subjects. One specimen (3 mL) was centrifuged at 1028 *g* for 15 minutes using a vacuum tube without anticoagulation (for testing 25-[OH]D, anti-CCP, and anti-MCV), the other (2 mL) was treated with Ethylene Diamine Tetraacetic Acid (EDTA)-K2 (for testing RDW). RDW was determined via the Mindray BC-5300 automatic blood cell analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd). 25-(OH)D was detected by the electrochemical luminescence method using a Roche cobas e 601 automatic electrochemical luminescence instrument and corresponding detection reagent (Roche Diagnostics Ltd.). Anti-CCP and anti-MCV were tested using enzyme-linked immunosorbent assay (ELISA), and the reagents were purchased separately. All actions were strictly in accordance with the instructions for the reagents or instrument, as well as the quality-management standards of the Second Affiliated Hospital of Nanchang University.

Statistical Methods

Statistical analyses were performed using Statistical Product and Service Solutions software, version 25.0 (IBM Corporation) and GraphPad Prism, version 8.0 (GraphPad Software) for chart mapping. We used Kolmogorov-Smirnov (K-s) testing for the normality of measurement data and Levene testing for the homogeneity of variance. The data of normal distribution were expressed as mean (SD) ($\bar{x}\pm s$), analysis of variance (ANOVA) was used for comparison among multiple groups, and least significant difference (LSD) *t* testing was used for pairwise comparison. Skewed distribution data were

DOI: 10.1093/labmed/lmaa040

represented by median and interquartile interval (P25–P75). We used nonparametric Kruskual-Wallis testing for comparison among multiple groups and Nemenyi testing for pairwise comparison.

We drew the receiver operating characteristic (ROC) curve and compared the AUCs of each indicator. In the joint evaluation, when all the evaluation indices are positive in a series, the result is positive, and when any one is positive in parallel, the result is also positive. $P < .05$ was considered to be statistically significant.

Results

Comparison of Basic Data for Patients in Each Group

Age and sex of the 3 groups were evaluated using ANOVA and χ^2 testing, respectively. The differences were not statistically significant ($P > .05$), as shown in **Table 1**.

Comparison of Test Results of Anti-MCV, Anti-CCP, RDW, and 25-(OH)D

Anti-CCP and anti-MCV levels in patients with RA were significantly higher than those in the disease controls and the healthy controls, as determined by Nemenyi testing; the differences were highly statistically significant ($P < .01$). RDW levels in patients with RA were significantly higher than those in healthy controls, via LSD *t* testing; the differences were statistically significant ($P < .05$), as shown in **Table 2**.

The ROC Curve Analyses of Anti-MCV, Anti-CCP, RDW, and 25-(OH)D

The AUC of anti-MCV, anti-CCP, RDW, and 25-(OH)D were 0.857, 0.890, 0.611, and 0.569, respectively. The AUC of anti-MCV and anti-CCP were larger than in other analytes, and their diagnostic value was relatively higher (**Figure 1**, **Table 3**).

Diagnostic Efficacy Evaluation of Anti-MCV, anti-CCP, RDW, and 25-(OH)D

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of anti-CCP and

Table 1. Comparison of Clinical Characteristics of Patients in Each Group

Characteristic	Group			F/χ^2	*P* Value
	RA	Disease Controls[a]	Healthy Controls		
No.	119	114	40		
Age, y, mean (SD)	50.90 (9.94)	51.65 (10.32)	50.83 (13.00)	1.609	.16
Sex, no. (male/female)	29/85	30/89	9/31	0.146	.93

RA, rheumatoid arthritis.
[a]*Includes 41 patients with systemic lupus erythematosus, 27 with Sjögren syndrome, 30 with arthritis, and 16 with ankylosing spondylitis.*

Table 2. Comparison of Test Results for Anti-MCV, Anti-CCP, RDW, and 25-(OH)D

Item	Group		
	RA	Disease Controls	Healthy Controls
Anti-CCP (U/ML), median (interquartile interval [P25–P75])	347.32 (36.22–1475.87)	11.23 (6.53–16.77)[a]	12.36 (7.14–19.04)[a]
Anti-MCV (U/ML), median (P25–P75)	268.07 (54.59–1000.00)	12.10 (8.22–18.21)[a]	12.37 (8.15 –18.92)[a]
RDW (%), mean (SD)	14.06 (1.43)	14.15 (2.98)	13.26 (1.57)[b]
25-(OH)D (μg/L), mean (SD)	28.14 (11.19)	24.16 (10.47)[c]	29.64 (9.66)

anti-MCV, anti–mutated citrullinated vimentin; anti-CCP, anti–citrullinated peptide; RDW, red blood cell distribution width; 25-(OH)D, 25-hydroxyvitamin D; RA, rheumatoid arthritis.
[a]*Compared with RA using Nemenyi testing;* P *<.01.*
[b]*Compared with RA by least significant difference* t *test,* P *<.05.*
[c]*Compared with RA by least significant difference* t *testing;* P *<.01.*

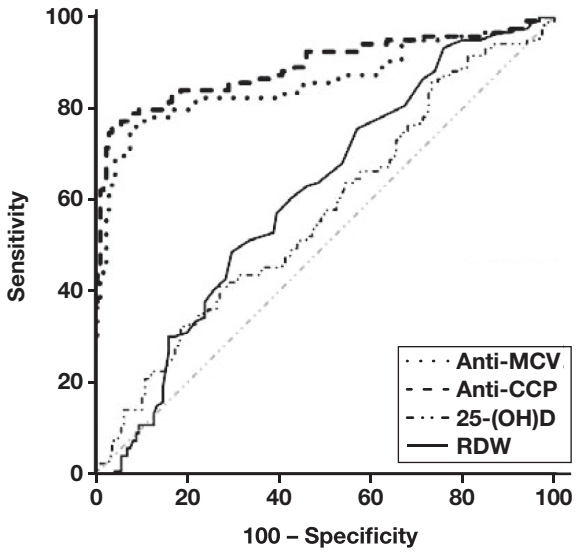


Figure 1

Specificity and sensitivity (per 100) of anti–mutated citrullinated vimentin (anti-MCV), anti-citrullinated peptide (anti-CCP), 25-hydroxyvitamin D (25-[OH]D), and red blood cell distribution width (RDW).

Table 3. ROC Curve Analysis Results[a]

Analyte	AUC	Cutoff Value	*P* Value (95% CI)
Anti-MCV	0.857	55.32	<.001 (.81–.91)
Anti-CCP	0.890	33.40	<.001 (.85–.93)
RDW	0.611	13.85	.002 (.54–.68)
25-(OH)D	0.569	30.84	.049 (0.50–.64)

ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; anti-MCV, anti–mutated citrullinated vimentin; anti-CCP, anti–citrullinated peptide; RDW, red blood cell distribution width; 25-(OH)D, 25-hydroxyvitamin D; RA, rheumatoid arthritis.

[a]*In total, 119 patients with RA were considered to be the disease group. The 114 patients without RA, combined with the 40 healthy people, were considered to be the no-disease group.*

anti-MCV were higher than in RDW and 25-(OH)D. These values are shown in **Table 4**.

Combined Detection of Anti-MCV, Anti-CCP, RDW, and 25-(OH)D

When RDW, 25-(OH)D, anti-CCP, and anti-MCV were connected in a series, the sensitivity was the lowest. The highest sensitivity shown was 94.1%, which occured when RDW, 25-(OH)D, and anti-CCP or RDW, 25-(OH)D, anti-CCP, and anti-MCV were connected in parallel, as shown in **Table 5**.

Discussion

RA is a chronic, progressive, systemic autoimmune disease, which can involve the heart, lungs, blood, nerves, and other organs and tissues; the symptoms are aggravated as the disease course progresses. The etiology of RA has not been fully understood so far. Patients with RA account for 1% to 2% of the worldwide population, among whom the number of female patients is approximately 3 times that of male patients. Also, the disability rate within 5 to 10 years of contracting the disease is as high as 60%. Early diagnosis and treatment are the keys to controlling the continued deterioration that results from RA disease and reducing the disability rate.

Rheumatoid factor (RF) is a common diagnostic index for clinical diagnosis of RA. However, RF can also appear in other autoimmune and infectious diseases; in a few healthy people, its specificity is low. Therefore, it is particularly important to search for the diagnostic indices with high diagnostic value or the joint detection of existing indices. It had been reported in the literature[2,3] that anti-CCP is a highly specific indicator for early diagnosis of RA and has high clinical diagnostic value. In 2010, it was included in the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) diagnostic criteria.

Anti-MCV has been a highly researched topic within RA research in recent years. Study results[10,11] have shown that anti-MCV has higher diagnostic efficiency than other analytes. Also, it has been shown[12,13] that RDW had a certain correlation with the occurrence and development of RA.

In recent years, the number of studies on RA has gradually increased.[12,13] As the main metabolic form of Vitamin D, 25-(OH)D is the best indicator to evaluate Vitamin D level in the body. The serum level of 25-(OH)D in patients with RA was generally reduced and correlated with disease activity. It has also been reported[14] that lack of 25-(OH)D in patients for RA was an important factor in disease progression.

Anti-MCV, anti-CCP, RDW, and 25-(OH)D were detected in patients with RA, disease controls, and healthy controls in our study. The results showed that the RDW levels of patients with RA were higher than those in healthy controls. We analyzed the reasons for the elevation of RDW in patients. Those levels were affected by many factors, among which oxidative stress and

DOI: 10.1093/labmed/lmaa040

Table 4. Clinical Evaluation Results of Anti-MCV, Anti-CCP, RDW, and 25-(OH)D

Analyte	Sens (%)	Spec (%)	YI	+LR	–LR	PPV (%)	NPV (%)
Anti-MCV	75.6	92.2	0.678	9.69	0.26	88.2	83.0
Anti-CCP	76.5	96.1	0.726	19.62	0.25	93.8	84.1
RDW	48.7	70.1	0.188	1.63	0.73	55.7	63.9
25-(OH)D	41.2	72.7	0.138	1.51	0.81	53.8	61.5

anti-MCV, anti–mutated citrullinated vimentin; anti-CCP, anti–citrullinated peptide; RDW, red blood cell distribution width; 25-(OH)D, 25-hydroxyvitamin D; Sens, sensitivity; Spec, specificity; YI, Youden index; +LR, positive likelihood ratio; –LR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

Table 5. Clinical Evaluation of Mutual Index Combination Test

Combined Detection	In Series					In Parallel				
	Sens (%)	Spec (%)	YI	+LR	–LR	Sens (%)	Spec (%)	YI	+LR	–LR
RDW + anti-CCP	37.8	97.4	0.352	14.54	0.64	87.4	68.8	0.562	2.80	0.18
RDW + anti-MCV	38.7	97.4	0.361	14.88	0.63	85.7	64.9	0.506	2.44	0.22
25-(OH)D + anti-CCP	30.3	99.4	0.297	50.50	0.70	87.4	70.1	0.575	2.92	0.18
25-(OH)D + anti-MCV	30.3	97.4	0.277	11.65	0.72	87.4	67.5	0.549	2.69	0.19
Anti-CCP + anti-MCV	72.3	98.7	0.71	55.61	0.28	80.7	89.6	0.703	7.76	0.22
RDW + 25-(OH)D + anti-CCP	14.3	100.0	0.143	…	0.86	94.1	50.0	0.441	1.88	0.12
RDW + 25-(OH)D + anti-MCV	14.3	99.4	0.137	23.83	0.86	93.3	47.4	0.407	1.77	0.14
RDW + anti-CCP + anti-MCV	36.1	98.7	0.348	27.77	0.65	88.2	63.6	0.518	2.42	0.19
25-(OH)D + anti-CCP + anti-MCV	27.7	100.0	0.277	…	0.72	89.1	65.6	0.547	2.59	0.17
RDW + 25-(OH)D + anti-CCP + anti-MCV	13.4	100.0	0.134	…	0.87	94.1	46.8	0.409	1.77	0.13

Sens, sensitivity; Spec, specificity; YI, Youden index; +LR, positive likelihood ratio; –LR, negative likelihood ratio; RDW, red blood cell distribution width; anti-CCP, anti–citrullinated peptide; anti-MCV, anti–mutated citrullinated vimentin; 25-(OH)D, 25-hydroxyvitamin D; … , nonapplicable.

inflammation were the decisive factors affecting RDW. Lee WS et al[15] also confirmed that RDW in patients with RA was correlated with inflammatory markers; this finding suggests that the reason for the increase of RDW may be related to inflammatory response in patients with RA. When we compared 25-(OH)D levels in patients with RA and in healthy controls, the difference was not statistically significant. However, the results reported by Abourazzak et al[16] showed that the level of 25-(OH)D in patients with RA was lower than that in healthy people, which was inconsistent with the results of this study. A possible reason for those differing results may be different regions, living environments, and detection reagents. The results of this study showed that anti-CCP and anti-MCV levels in patients with RA were significantly higher than those in patients without RA and healthy controls. These findings suggest that anti-MCV and anti-CCP may have clinical value in the differential diagnosis of RA.

By comparing the AUCs of anti-MCV, anti-CCP, RDW, and 25-(OH)D from the results of this study, we discovered that the AUC of anti-CCP was the largest, indicating that its diagnostic value was higher. Meanwhile, our results showed that the sensitivity and specificity of anti-CCP were also relatively higher (76.5% and 96.1%, respectively), which was basically consistent with the results reported by Cui et al[17] and Lin Y-T et al.[18] The results of diagnostic performance evaluation showed that the PPV of anti-CCP was the highest among the 4 indicators, which suggested that anti-CCP could be used as one of the main serological indicators for the differential diagnosis of RA. The Youden index of anti-CCP, at 0.726, was the largest when tested by itself. Among the 4 indicators, the authenticity was the strongest, so it was more accurate in distinguishing patients with RA and healthy people. The sensitivity and specificity of anti-MCV were 75.6% and 92.2%, respectively, and its diagnostic efficacy was lower than that of anti-CCP, which was basically consistent with the research results of Lee YH et al.[19]

Study results[20] have shown that the sensitivity and specificity of anti-MCV were 82% and 95%, respectively; these values were higher than the results from this study, which may be related to the selection of disease control group and other factors. Anti-MCV is a citrulline-related autoantibody,

which exists in synovial fluid, synovial cells, and the serum of patients with RA[21]: it has high sensitivity in RA diagnosis. The AUC of anti-MCV was 0.857, and its diagnostic value was relatively high. Compared with RDW and 25-(OH)D, anti-MCV had a higher Youden index value. The AUC of RDW and 25-(OH)D was smaller, and the differential diagnostic value was lower than anti-CCP and anti-MCV. However, RDW and 25-(OH)D still had diagnostic value, indicating that they could be used as serological auxiliary indicators for the diagnosis of RA.

Although the diagnostic values of anti-MCV and anti-CCP were relatively high among the 4 indicators, they are still insufficient in the clinical diagnosis of RA. Therefore, the clinical evaluation of the mutual index combination test was required. When RDW, 25-(OH)D, and anti-CCP were combined in parallel, the sensitivity was highest (92.4%)—higher than single detection—and significantly reduced the rate of missed diagnosis of RA. Our results showed that the largest specificity was 100% when RDW, 25-(OH)D, and anti-CCP; 25-(OH)D, anti-CCP, and anti-MCV; or RDW and 25-(OH)D were connected in series, and it was higher than the specificity of single testing, which greatly reduced the misdiagnosis rate of RA. When these biomarkers were combined in series, specificity was improved and sensitivity was reduced. Also, in parallel, sensitivity was improved and specificity was decreased. Therefore, the appropriate joint detection method should be determined according to the specific situation in clinical practice.

In summary, anti-CCP and anti-MCV are ideal indicators for the diagnosis of RA. Also, RDW and 25-(OH)D can be used as auxiliary diagnostic indicators. The combination of these 4 indicators can improve the level of diagnosis, as well as the sensitivity and specificity, all of which contribute to the differential diagnosis of RA. **LM**

Acknowledgments: This work was supported by the National Natural Science Foundation of China (81760382) and Key Research and Development Projects of Jiangxi Province (20192BBG70033).

References

1. McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet.* 2017;389(10086):2328–2337.
2. Tan EM, Smolen JS. Historical observations contributing insights on etiopathogenesis of rheumatoid arthritis and role of rheumatoid factor. *J Exp Med.* 2016;213(10):1937–1950.
3. Kedar MP, Acharya RV, Prakashini K. Performance of the 2010 American College of Rheumatology/European League against Rheumatism (ACR/EULAR) criteria for classification of rheumatoid arthritis in an Indian population: An observational study in a single centre. *Indian J Med Res.* 2016;144(2):288–292.
4. Barouta G, Katsiari CG, Alexiou I, et al. Anti-MCV antibodies predict radiographic progression in Greek patients with very early (<3 months duration) rheumatoid arthritis. *Clin Rheumatol.* 2017;36(4):885–894.
5. Yunchun L, Yue W, Jun FZ, Qizhu S, Liumei D. Clinical significance of red blood cell distribution width and inflammatory factors for the disease activity in rheumatoid arthritis. *Clin Lab.* 2016;62(12):2327–2331.
6. Lee YH, Bae S-C. Vitamin D level in rheumatoid arthritis and its correlation with the disease activity: a meta-analysis. *Clin Exp Rheumatol.* 2016;34(5):827–833.
7. Lin J, Liu J, Davies ML, Chen W. Serum vitamin D level and rheumatoid arthritis disease activity: review and meta-analysis. *PLoS One.* 2016;11(1):e0146351.
8. Atwa MA, Balata MG, Hussein AM, Abdelrahman NI, Elminshawy HH. Serum 25-hydroxyvitamin D concentration in patients with psoriasis and rheumatoid arthritis and its association with disease activity and serum tumor necrosis factor-alpha. *Saudi Med J.* 2013;34(8):806–813.
9. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 2010;62(9):2569–2581.
10. Nass FR, Skare TL, Goeldner I, Nisihara R, Messias-Reason IT, Utiyama SRR. Analysis of four serum biomarkers in rheumatoid arthritis: association with extra articular manifestations in patients and arthralgia in relatives. *Rev Bras Reumatol Engl Ed.* 2017;57(4):286–293.
11. Meyer PWA, Ally MTM, Hodkinson B, Anderson R, Tikly M. Comparison of the diagnostic potential of three anti-citrullinated protein antibodies as adjuncts to rheumatoid factor and CCP in a cohort of South African rheumatoid arthritis patients. *Rheumatol Int.* 2018;38(6):993–1001.
12. Tecer D, Sezgin M, Kanık A, et al. Can mean platelet volume and red blood cell distribution width show disease activity in rheumatoid arthritis? *Biomark Med.* 2016;10(9):967–974.
13. He Y, Liu C, Zeng Z, Ye W, Lin J, Ou Q. Red blood cell distribution width: a potential laboratory parameter for monitoring inflammation in rheumatoid arthritis. *Clin Rheumatol.* 2018;37(1):161–167.
14. Zakeri Z, Sandoughi M, Mashhadi MA, Raeesi V, Shahbakhsh S. Serum vitamin D level and disease activity in patients with recent onset rheumatoid arthritis. *Int J Rheum Dis.* 2016;19(4):343–347.
15. Lee WS, Kim T-Y. Relation between red blood cell distribution width and inflammatory biomarkers in rheumatoid arthritis. *Arch Pathol Lab Med.* 2010;134(4):505–506.
16. Abourazzak FE, Talbi S, Aradoini N, Berrada K, Keita S, Hazry T. 25-Hydroxy vitamin D and its relationship with clinical and laboratory parameters in patients with rheumatoid arthritis. *Clin Rheumatol.* 2015;34(2):353–357.
17. Cui J, Taylor KE, Lee YC, et al. The influence of polygenic risk scores on heritability of anti-CCP level in RA. *Genes Immun.* 2014;15(2):107–114.
18. Lin Y-T, Tsai T-H. Anti-CCP antibody in a patient with generalized discoid lupus erythematosus, vasculitis and acute interstitial lung disease but not rheumatoid arthritis. *J Eur Acad Dermatol Venereol.* 2014;28(6):826–828.
19. Lee YH, Bae S-C, Song GG. Diagnostic accuracy of anti-MCV and anti-CCP antibodies in rheumatoid arthritis: A meta-analysis. *Z Rheumatol.* 2015;74(10):911–918.
20. Degboé Y, Constantin A, Nigon D, et al. Predictive value of autoantibodies from anti-CCP_2, anti-MCV and anti-human citrullinated fibrinogen tests, in early rheumatoid arthritis patients with rapid radiographic progression at 1 year: results from the ESPOIR cohort. *RMD Open.* 2015;1(1):e000180.
21. van Heemst J, Trouw LA, Nogueira L, et al. An investigation of the added value of an ACPA multiplex assay in an early rheumatoid arthritis setting. *Arthritis Res Ther.* 2015;17:276.

DOI: 10.1093/labmed/lmaa040

The Association Between Serum Leptin Levels and Cardiovascular Events in Patients with Rheumatoid Arthritis

Jiliang Chen, MD,[1] Zhiping Xie, PhD,[2] Zou Bin, MD[1]*

Laboratory Medicine 2021;52:86-92

DOI: 10.1093/labmed/lmaa037

ABSTRACT

Objective: Cardiovascular diseases (CVDs) are important complications for patients with rheumatoid arthritis (RA). The study aimed to explore whether serum leptin is associated with a increased risk of cardiovascular (CV) events in patients with RA.

Methods: Two hundred twenty-three patients with RA were followed for a mean of 40 (range = 8-42) months. Serum leptin levels were measured at baseline. Cox regression analysis was performed to assess the association between leptin levels and the risk of CV events.

Results: The univariate analysis showed that patients with RA with higher serum leptin levels had higher rates of CV events and CV mortality, respectively (P <.001). The logistic regression model showed that leptin was independently related to CVD history (odds ratio = 1.603, 95% confidence interval [CI], 1.329–2.195; P =.005) after adjusting for confounding factors in patients with RA at baseline. The multivariate Cox proportional hazard model suggested that leptin was an independent prognostic factor for CV events in patients with RA after adjustments were made for clinical confounding factors (hazard ratio = 2.467, 95% CI, 2.019–4.495; P <.001). The Kaplan-Meier analysis showed that compared with patients with RA with leptin levels below the median value (≤15.4 mg/L), patients with leptin above the median value (>15.4 μg/L) had a higher rate of CV events (P <.001).

Conclusion: Leptin was significantly associated with CV events in patients with RA. Elevated serum leptin levels may be a reliable prognostic factor for predicting CV complications in patients with RA.

Keywords: leptin, rheumatoid arthritis, cardiovascular event, prognostic value

Rheumatoid arthritis (RA) has become an important public health problem because of its high disability rate and huge social burden in the world.[1,2] The rate of cardiovascular diseases (CVDs) in patients with RA is significantly higher than that in the general population without RA, and CVDs have become the leading cause of death in patients with RA.[3,4] Although the exact underlying mechanisms are still unclear, increasing inflammation and oxidative stress in RA are regarded as the primary mechanisms causing a high risk for cardiovascular (CV) complications.[5,6]

Leptin is a kind of hormone secreted by adipose tissue.[7] It is generally believed that leptin participates in the regulation of sugar, fat, and energy metabolism after entering the blood circulation.[8,9] Increased leptin levels will lead to less appetite and more energy expenditure by the hypothalamus in the general population.[8-11] However, many studies have also indicated that leptin is involved in the regulation of immune responses. It can promote the secretion of many proinflammatory cytokines in vivo and in vitro and plays an important role in inflammatory responses.[12-16] As a chronic inflammatory disease, RA promotes the development of various CVD-related pathological mechanisms.[17-19] Given the close association between leptin and inflammation, we hypothesized that leptin may be associated with CV outcomes in patients with RA.

Abbreviations:

CVDs, cardiovascular diseases; RA, rheumatoid arthritis; CV, cardiovascular; CI, confidence interval; HF, heart failure; BMI, body mass index; SCr, serum creatinine; BUN, serum urea nitrogen; FBG, fasting blood glucose; ALB, albumin; hs-CRP, high-sensitivity C-reactive protein; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; Hb, hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; BP, blood pressure; OR, odds ratio.

[1]Department of Orthopedics, The Affiliated Mindong Hospital of Fujian Medical University, Ningdei, Fu Jian, People's Republic of China, [2]Department of Neurosurgery, The First Affiliated Hospital of Nanchang University, Nanchang Jiangxi, People's Republic of China

*To whom correspondence should be addressed.
zoubin09@qq.com

There have been no relevant studies to assess the association between serum leptin and CV events in patients with RA. The aim of this study was to investigate whether elevated leptin levels contribute to the increased risk for CV events during a follow-up period (3 years).

Materials and Methods

Study Population

Between January 2014 and October 2015, we studied 223 patients with RA from the National Population Health Science Data Center in China. This database contains the original data of various medical industries in China, so this study has sufficient data to be analyzed. These patients were stable without other serious illnesses for more than 2 months before hospital admission. The diagnosis of RA was confirmed by 2 rheumatologists according to the 2010 American College of Rheumatology/European League Against Rheumatism Classification Criteria for RA.[20] The main reasons for the hospitalization of these patients with RA were extra-articular complications and/or self-required hospitalization because of their older age (median age = 68.1 years). All patients were treated with regular anti-RA therapy during hospitalization. Patients with neoplastic diseases, acute kidney disease or dialysis, serious liver diseases, or other serious diseases not associated with RA-related complications were excluded. Nine patients were excluded from the study because of severe liver and other malignant diseases. For the purposes of this study, the diagnosis of CVD history included myocardial infarction, heart failure (HF), stroke, and peripheral vascular disease. All clinical characteristic data on this study, including age, sex, body mass index (BMI), and history of smoking, drinking, and CVD, were obtained from patients' current or previous medical records in our hospital.

All included patients were followed up for a mean period of 40 months (range = 8-42 months). These patients with RA were followed for time-to-event analysis until occurrence of CV events (fatal and nonfatal events). The nonfatal CV events consisted of myocardial infarction, stroke, HF, and peripheral vascular disease. The fatal events were defined as CV death caused by myocardial infarction, stroke, HF, peripheral vascular disease, and sudden cardiac death. The diagnosis of all CV events was confirmed by the 2 attending physicians. These follow-up data were recorded by routine outpatient clinic visits and telephone contacts. According to Declaration of Helsinki guidelines, the Ethics Committee of the Affiliated Mindong Hospital of Fujian Medical University, Fu Jian, People's Republic of China, approved this prospective study, and all patients in our study gave written informed consent.

Measurement of Leptin

Venous blood specimens from patients with RA were collected the first morning after admission. The specimens were immediately prepared by centrifugation and processed with cryopreservation (–80°C) for testing leptin levels. Serum specimens were assayed in duplicate using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN: Elisa Development System, Duoset Human Leptin, Catalog No. DY398) for measuring leptin levels. This assay measured the total amount of leptin present in a specimen, independent of the presence of leptin-binding proteins. The detection limit was 0.1 μg/L. The intra-assay variation was <3.5%, and the interassay variation was <6.4%, which suggested that the test method was reliable for the detection of leptin levels.

Laboratory Measurements and Definitions

The blood specimens collected from patients with RA the first morning after admission were also used for measuring serum creatinine (SCr), serum urea nitrogen (BUN), fasting blood glucose (FBG), albumin (ALB), high-sensitivity C-reactive protein (hs-CRP), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) with the use of the Siemens ADVIA 2400 automatic biochemistry analyzer (Siemens AG). Hemoglobin (Hb) was obtained by using an automated blood counter (Sysmex XE5000; Emilio de Azevedo Campos, Porto, Portugal). The homeostasis model assessment of insulin resistance (HOMA-IR) was used to calculate insulin resistance.

Statistical Analyses

All of the analyses were performed by using SPSS 23.0, and $P \leq .05$ was statistically significant. Normality of data

DOI: 10.1093/labmed/lmaa037

was analyzed by the Kolmogorov-Smirnov test combined with Q-Q plots. The data that were not normally distributed were expressed by median (interquartile range) and were analyzed by the Mann-Whitney *U* test. Data were presented as mean ± standard deviation for normally distributed data and were analyzed by independent *t*-test. The distributions of categorical variables were studied by using the χ^2 test. The associations of serum leptin levels with CVD history and CV events were analyzed by logistic regression and Cox proportional hazard analysis, respectively. The multivariate logistic regression analysis was performed to identify the independent association between serum leptin levels and CVD history in patients with RA at baseline. The Cox proportional hazard model was used to identify the independent prognostic factors for CV events. In the Cox proportional hazard analysis, the factors (P <.1) performed by univariate analysis were entered into the multivariate analysis. In addition, we also adjusted for clinical data relevant to CVDs even if these were not significantly associated with outcomes in the univariate analysis. The CV events-free curves were constructed according to the Kaplan-Meier method and were compared using the log-rank test.

Results

Clinical Characteristics

Clinical data for all study participants are presented in Table 1. The median serum leptin level in this cohort study was 15.4 (6.4–25.3) μg/L. We stratified the study cohort into 2 groups according to median leptin level. For clinical features, there were no significant differences in age, sex, admission blood pressure (BP; systolic BP and diastolic BP) between the 2 groups. Patients with RA with leptin levels above the median (>15.4 μg/L) had a higher BMI, a history of smoking and drinking, and significantly higher percentages of CVD history, CV events, and CV mortality. For laboratory measurements, the levels of TC, TG, LDL, FBG,

Table 1. Clinical Characteristics of Patients with RA

Variables	All Patients (n = 223)	Below-Median Leptin (≤15.4 μg/L, n = 112)	Above-Median Leptin (>15.4 μg/L, n = 111)	*P* Value
Age (y)	68.1 (57.2–75.6)	67.5 (54.1–72.3)	69.3 (58.9–78.6)	.103
Sex (men), n (%)	115 (51.6)	59 (52.7)	53 (47.7)	.227
BMI (kg/m^2)	26.1 (24.6–27.1)	25.4 (23.4–26.2)	27.8 (25.6–28.5)	<.001
Smoking history, n (%)	48 (21.5)	19 (17.0)	29 (26.1)	.001
Drinking history, n (%)	53 (23.8)	20 (17.9)	33 (20.7)	.002
CVD history, n (%)	19 (12.4)	4 (3.6)	15 (13.5)	<.001
CV events, n (%)	43 (19.3)	10 (8.9)	33 (29.7)	<.001
CV mortality, n (%)	12 (5.4)	2 (1.8)	10 (9.0)	<.001
Admission systolic BP, mm Hg	127.4 (110.4–145.0)	126.7 (108.4–144.2)	128.2 (111.3–147.5)	.237
Admission diastolic BP, mm Hg	78.8 (71.2–86.5)	77.3 (68.3–80.2)	79.6 (76.7–89.4)	.130
Laboratory measurements				
FBG, mmol/L	5.97 (4.86–8.30)	5.78 (4.41–7.25)	6.31 (5.09–8.94)	.029
HOMA-IR	2.69 ± 1.78	2.48 ± 1.53	3.34 ± 1.93	<.001
TC, mmol/L	3.87 ± 1.22	3.78 ± 0.98	4.42 ± 1.34	.003
TG, mmol/L	1.79 ± 0.43	1.37 ± 0.39	2.01 ± 0.49	.002
HDL, mmol/L	0.82 (0.66–1.03)	0.81 (0.63–1.04)	0.89 (0.81–1.09)	.092
LDL, mmol/L	2.43 (1.64–2.78)	2.15 (1.58–2.50)	2.58 (1.86–3.60)	.003
Hb, g/L	124.7 (105.3–139.5)	123.9 (103.2–137.4)	125.8.0 (107.0–141.3)	.207
ALB, g/L	38.5 (36.9–42.4)	40.3 (35.3–41.7)	37.8 (38.4–42.9)	<.001
hs-CRP, mg/L	7.40 (2.13–26.78)	6.28 (1.92–22.14)	9.36 (2.29–27.20)	.035
SCr, umol/L	88.7 ± 8.2	88.4 ± 7.9	89.3 ± 8.7	.347
BUN, mmol/L	5.6 ± 2.4	5.0 ± 2.1	5.8 ± 2.7	.181

RA, rheumatoid arthritis; BMI, body mass index; CV, cardiovascular; CVD, cardiovascular disease; BP, blood pressure; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; Hb, hemoglobin; ALB, albumin; hs-CRP, high-sensitivity C-reactive protein; SCr, serum creatinine; BUN, serum urea nitrogen.
Data are presented as mean ± SD for normally distributed data, as median (interquartile range) for nonnormally distributed data, and as n (%) for categoric variables.

HOMA-IR and hs-CRP were higher and ALB levels were lower in patients with higher leptin levels. There were no significant differences in Hb, SCr, BUN, and HDL between the 2 groups.

Serum Leptin Showed an Independent Association with CVD History

To determine the association of serum leptin levels in patients with RA with a history of CVD, multivariate logistic regression analyses were performed (**Table 2**). The Crude Model indicated that higher serum leptin levels were associated with a higher risk of CVD history after adjusting for age and sex. After adjustments were made for age, sex, BMI, smoking history, drinking history, and admission systolic BP and diastolic BP, the results in Model 1 were similar to those of the Crude Model. This association remained statistically significant and changed little when we continued to add laboratory measurements to Model 1 (to formulate Model 2). The fully adjusted odds ratio (OR) of CVD history in Model 2 was 1.603 (1.329–2.195) in serum levels in quartile 4 (the highest) vs in quartile 1 (the lowest).

Serum Leptin Levels Showed an Independent Value for Predicting CV Events in Patients with RA by Cox Proportional Hazard Regression Analysis

We determined CV events from patient inclusion onward with a mean follow-up period of 40 (range = 8-42) months (**Table 3**). Fifteen patients died, and 12 of those deaths were related to CV causes including myocardial infarction (n = 5), HF (n = 3), stroke (n = 2), complicated peripheral vascular disease (n = 1), and sudden cardiac death (n = 1). Three patients died of non-CV causes. In addition, a total of 31 nonfatal CV events took place during the follow-up period. These nonfatal CV events included myocardial infarction (n = 11), HF (n = 8), stroke (n = 10), and peripheral vascular disease (n = 2).

The predictors for CV events (fatal and nonfatal CV events = 43) were analyzed by univariate and multivariate Cox regression. The univariate Cox analysis showed that age, BMI, smoking history, drinking history, CVD history, TC, TG, LDL, ALB, hs-CRP, HOMA-IR, and leptin were each significantly associated with an increased risk of CV events (**Table 3**). After adjustments were made for age, BMI, smoking history, drinking history, CVD history, systolic BP, HOMA-IR, TC, TG, LDL, ALB, hs-CRP, and SCr, the multivariate Cox analysis showed that higher leptin levels were still significantly associated with an increased risk of CV events (OR = 2.467, 95% confidence interval, 2.019–4.495; *P* <.001). In addition, Kaplan-Meier survival curves showed that patients with leptin levels above the median value (>15.4 μg/L) had a higher rate of CV events than did patients with leptin levels below the median value (≤15.4 μg/L; log-rank test, *P* <.001; **Figure 1**).

We performed a sensitivity analysis to assess the association of serum leptin levels with CV events in patients with RA who did not have a previous CVD history (n = 204). The number of CV events was 36. The multivariate Cox analysis showed that higher leptin levels were still independently associated with an increased risk of CV events (data not shown). Similarly, compared with patients with leptin levels below the median value, Kaplan-Meier survival curves still showed that patients with leptin levels above the median value had a higher rate of CV events (log-rank test, *P* <.001; data not shown).

Table 2. Logistic Regression Analysis of the Relationship Between CVD History and Leptin in Patients with RA

Variables	Crude Model[a]	Model 1[b]	Model 2[c]
Serum leptin level			
Quartile 1 (low)	1.000 [Reference]	1.000 [Reference]	1.000 [Reference]
Quartile 2	1.235 (1.102–1.583)	1.200 (1.090–1.342)	1.116 (1.004–1.248)
Quartile 3	1.422 (1.225–1.704)	1.386 (1.201–1.652)	1.349 (1.184–1.523)
Quartile 4 (high)	1.739 (1.393–2.291)	1.689 (1.361–2.273)	1.603 (1.329–2.195)
P trend	<.001	<.001	.005

RA, rheumatoid arthritis; BMI, body mass index; CVD, cardiovascular disease; BP, blood pressure.
[a]*Crude model adjusted for age and sex.*
[b]*Model 1: Adjusted for age, sex, BMI, smoking history, drinking history, and admission systolic BP and diastolic BP.*
[c]*Model 2: Adjusted for age, sex, BMI, smoking history, drinking history, and laboratory measurements.*

Lab Medicine 2021;52;86–92
DOI: 10.1093/labmed/lmaa037

Table 3. Univariate and Multivariate Cox Proportional Hazard Analysis of Predicting CVD Events in Patients with RA

Variables	Univariate Cox			Multivariate Cox		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age (per 1-SD increase)	1.314	1.186–2.420	.017	1.226	1.122–2.018	.040
Sex	1.109	0.845–1.636	.349	…	…	…
BMI (kg/m^2)	1.758	1.231–2.682	.013	1.425	1.177–2.138	.019
Smoking history	1.341	1.024–1.743	.017	1.221	1.014–1.524	.044
Drinking history	1.201	1.042–1.802	.038	1.172	0.991–1.703	.059
History of CVDs	2.334	1.484–3.94	.009	1.883	1.402–3.75	.014
Systolic BP (per 1-SD increase)	1.305	0.543–1.802	.091	1.278	0.489–1.634	.104
Diastolic BP (per 1-SD increase)	1.100	0.876–1.632	.836	…	…	…
FBG (per 1-SD increase)	1.292	0.953–2.231	.119	…	…	…
HOMA-IR (per 1-SD increase)	1.613	1.124–2.428	.015	1.438	1.146–2.034	.042
TC (per 1-SD increase)	2.292	1.674–4.246	.002	1.935	1.521–4.037	.012
TG (per 1-SD increase)	2.051	1.232–3.591	.009	1.964	1.192–2.842	.011
HDL (per 1-SD increase)	0.897	0.932–1.214	.407	…	…	…
LDL (per 1-SD increase)	2.235	1.378–5.309	<.001	2.110	1.268–4.098	.009
Hb (per 1-SD increase)	1.202	0.912–1.702	.262	…	…	…
ALB (per 1-SD increase)	0.633	0.292–0.974	.046	0.921	0.634–1.303	.090
hs-CRP (per 1-SD increase)	1.407	1.007–2.781	.048	1.021	0.919–1.427	.207
SCr (per 1-SD increase)	1.313	0.893–2.301	.304	1.210	0.647–2.194	.410
BUN (per 1-SD increase)	1.029	0.934–1.587	.591	…	…	…
Serum leptin (per 1-SD increase)	2.812	2.178–5.246	<.001	2.467	2.019–4.495	<.001

RA, rheumatoid arthritis; BMI, body mass index; CV, cardiovascular; CVDs, cardiovascular diseases; BP, blood pressure; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; Hb, hemoglobin; ALB, albumin; hs-CRP, high sensitive C-reactive protein; SCr, serum creatinine; BUN, serum urea nitrogen; SD, standard deviation; CI, confidence interval.

Discussion

The new findings in this study: (i) serum leptin levels were independently and significantly related with CVD history in patients with RA at baseline, and (ii) higher leptin levels in patients with RA at baseline were directly associated with an increased risk of CV events during a 3-year follow-up.

A high rate of CV complications in patients with RA leads to poor prognosis.[3,4] Therefore, it is significant for clinicians to find risk factors of CV events and use them as targets for therapy or predictions for prognosis. Leptin, a hormone secreted from adipocytes, is involved in the regulation of energy expenditure and appetite.[10,11] Many studies have indicated that leptin participates in the regulation of immunity and inflammation responses. It can induce the secretion of tumor necrosis factor-a, interleukin-6, interleukin-1, and other proinflammatory cytokines in vivo and in vitro, which promotes systemic inflammation in the body.[12-16] Several clinical studies have also shown that leptin is significantly correlated with CVDs including coronary heart disease and stroke.[21-23] In addition, there is an association between leptin and oxidative stress: leptin has a facilitating role in oxidative stress, which causes the activation of inflammation in many organs or tissues.[24,25] Disproportionately increasing inflammation and oxidative stress play an important role in the occurrence and development of CV events in patients with RA.[3,4] In view of the potential regulatory role of leptin in inflammation and oxidative stress in CVD, our findings underscore that leptin is involved in the occurrence and development of CV events in patients with RA. Our results also showed that patients with RA with higher serum leptin levels had a higher rate of CV mortality during the follow-up period, consistent with the results of previous studies on the association of leptin with CVDs.[12–16,21-25] However, different from earlier research, our work included a composite endpoint, including myocardial infarction, stroke, peripheral vascular disease, and CV death, in patients with RA in a follow-up cohort study.

This study has some notable strengths. First, we found that serum leptin levels may be used as an effective prognostic

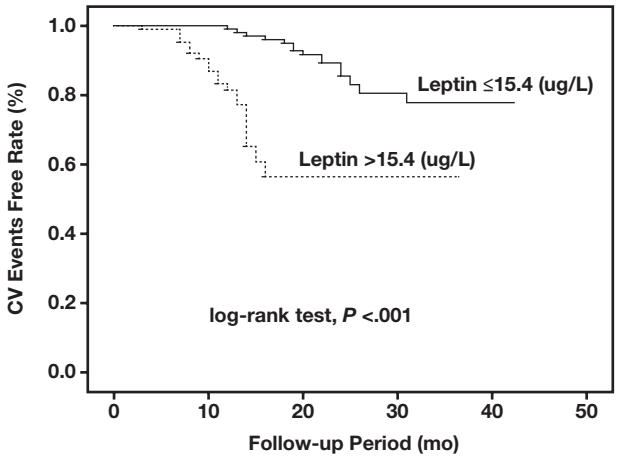


Figure 1

Kaplan-Meier analysis of CV events-free rate stratified into 2 groups by median levels of serum leptin. CV, cardiovascular.

factor for CV events in patients with RA, and the serum test is simple and convenient for patients in general. Second, we ensured comprehensive follow-up and rigorous adjudication of CV events, with more reliable data than have been generated in the literature. Sufficient confounding factors were corrected for the association between serum leptin levels and CV events.

The limitation of the study is that it is a single-center study with a small sample size. It is necessary for more multicenter and large-sample studies to be implemented to identify the association between serum leptin levels and CV outcomes in patients with RA in the future.

Conclusions

Leptin may provide a pathway linking RA to an increased risk of CV events. Serum leptin may be a reliable prognostic factor for predicting CVD in patients with RA. **LM**

References

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet.* 2010;376(9746):1094–1108.
2. Kitas GD, Gabriel SE. Cardiovascular disease in rheumatoid arthritis: state of the art and future perspectives. *Ann Rheum Dis.* 2011;70(1):8–14.
3. Crowson CS, Liao KP, Davis JM III, et al. Rheumatoid arthritis and cardiovascular disease. *Am Heart J.* 2013;166(4):622–628.e1.
4. Yousof M, Omar A, Elsayed H, et al. Cardiovascular diseases among Egyptian patients with rheumatoid arthritis and its relation to traditional risk factors: PP.2.82. *J Hypertens.* 2010;28(E Suppl A):1.
5. Zafari P, Zarifian A, Alizadeh-Navaei R, et al. Asymmetric and symmetric dimethylarginine concentration as an indicator of cardiovascular diseases in rheumatoid arthritis patients: a systematic review and meta-analysis of case-control studies. *Clin Rheumatol.* 2020;39(1):127–134.
6. Peters MJ, van Halm VP, Voskuyl AE, et al. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. *Arthritis Rheum.* 2009;61(11):1571–1579.
7. Lee M, Lee E, Jin SH, et al. Leptin regulates the pro-inflammatory response in human epidermal keratinocytes. *Arch Dermatol Res.* 2018;310(4):351–362.
8. Farooqi IS, Keogh JM, Kamath S, et al. Partial leptin deficiency and human adiposity. *Nature.* 2001;414(6859):34–35.
9. Benzler J, Andrews ZB, Pracht C, et al. Hypothalamic WNT signalling is impaired during obesity and reinstated by leptin treatment in male mice. *Endocrinology.* 2013;154(12):4737–4745.
10. Stanik J, Kratzsch J, Landgraf K, et al. Concordance of bioactive vs. total immunoreactive serum leptin levels in children with severe early onset obesity. *PLoS One*. 2017;12(5):e178107.
11. Kluge R, Scherneck S, Schurmann A, et al. Pathophysiology and genetics of obesity and diabetes in the New Zealand obese mouse: a model of the human metabolic syndrome. *Methods Mol Biol*. 2012;93359–93373.
12. Bulló M, García-Lorda P, Megias I, et al. Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. *Obes Res*. 2003;11(4):525–531.
13. Aboutaleb N, Zarrati M, Cheshmazar E, et al. Association between the circulating leptin levels and the biomarkers of oxidative stress and inflammation among Iranian overweight and obese adults. *Med J Islam Repub Iran*. 2017:3181.
14. Tazawa R, Uchida K, Fujimaki H, et al. Elevated leptin levels induce inflammation through IL-6 in skeletal muscle of aged female rats. *BMC Musculoskelet Disord.* 2019;20(1):199.
15. Żelechowska P, Agier J, Różalska S, Wiktorska M, Brzezińska-Błaszczyk E. Leptin stimulates tissue rat mast cell pro-inflammatory activity and migratory response. *Inflamm Res.* 2018;67(9):789–799.
16. Inoue W, Poole S, Bristow AF, Luheshi GN. Leptin induces cyclooxygenase-2 via an interaction with interleukin-1beta in the rat brain. *Eur J Neurosci.* 2006;24(8):2233–2245.
17. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology*. 2007;132(6):2169–2180.
18. Nishimura S, Manabe I, Nagasaki M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med.* 2009;15(8):914–920.
19. Martin SS, Qasim A, Reilly MP. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. *J Am Coll Cardiol.* 2008;52(15):1201–1210.
20. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 2010;62(9):2569–2581.
21. Wallace AM, McMahon AD, Packard CJ, et al. Plasma leptin and the risk of cardiovascular disease in the West of Scotland Coronary Prevention Study (WOSCOPS). *Circulation.* 2001;104(25):3052–3056.

DOI: 10.1093/labmed/lmaa037

22. Saber H, Himali JJ, Shoamanesh A, et al. Serum leptin levels and the risk of stroke: the Framingham Study. *Stroke.* 2015;46(10):2881–2885.

23. Jiménez I, Sobrino T, Rodríguez-Yáñez M, et al. High serum levels of leptin are associated with post-stroke depression. *Psychol Med.* 2009;39(7):1201–1209.

24. Bouloumie A, Marumo T, Lafontan M, Busse R. Leptin induces oxidative stress in human endothelial cells. *FASEB J.* 1999;13(10):1231–1238.

25. Bełtowski J, Wójcicka G, Jamroz A. Leptin decreases plasma paraoxonase 1 (PON1) activity and induces oxidative stress: the possible novel mechanism for proatherogenic effect of chronic hyperleptinemia. *Atherosclerosis.* 2003;170(1):21–29.

Management & Administration

Assessment of Medical Laboratory Undergraduates Training in Different School Year Systems

Yajun Lu, MM,[#] *Qianfeng Xia, MD,*[#] *Jun Yang, MPA*[*]

Laboratory Medicine 2021;52:93-101

DOI: 10.1093/labmed/lmaa033

ABSTRACT

Objective: To assess the effect of the change from the 5-year system of coursework to a 4-year system on the achievements of medical laboratory undergraduates.

Methods: We analyzed and then compared the topics of training and the test scores among all subject individuals in the 5-year vs the 4-year undergraduate program.

Results: Five-year-program undergraduates and 4-year-program undergraduates were required to complete 50 courses. The average weekly education time in the 4-year program (27.05 lesson-hours/week) was greater than that in the 5-year program (22.99 lesson-hours/week). The proportion of clinical practice in the hospital setting in the 4-year program (26.8%) was higher than that in the 5-year program (24.5%). The average, excellent, and good scores among 4-year-program undergraduates in general education courses, professional basic courses, professional courses, all courses, and common courses were lower than those scores among the 5-year-program undergraduates.

Conclusions: The 5-year undergraduate program should be adapted to help boost the achievements and practical skills among its students, in helping them adapt quickly to the new, 4-year training plan (which presented a serious challenge in our cohort).

Keywords: achievements, 5-year system, 4-year system, medical laboratory, curriculum composition, medical education

Undergraduate medical laboratory education was first established in China in 1984. Since then, medical laboratory higher education has gradually developed into an educational system with clear objectives, complete levels, and diverse forms. Before 2013, the training mode of the medical laboratory specialty was the 5-year system; on completion, a bachelor's degree in medicine was awarded.

The aim of the new 4-year medical laboratory training program is to equip technicians with skills suitable for clinical needs and the medical service.[1] Such a program is necessary to train undergraduates to master professional knowledge, theory, and skills in medical laboratory science. Qualified technicians can analyze and solve professional problems, as well as having the necessary competence for medical laboratory work in hospitals, blood supply stations, and other departments.

Compared with the 5-year system, the new 4-year system is facing numerous challenges, such as lack of experience in curriculum setting, short training time, and urgent tasks.[2] Implementing it on a broad scale would influence training direction, curriculum, employment prospects, qualification examination after graduation, continuing education, and so forth. 5-year program undergraduates were awarded degrees of Bachelor of Medicine, and 4-year program undergraduates were awarded degrees of Bachelor of Science. The new training program needs to be tested in practice, to prove whether it conforms to historical and modern development trends, as well as whether it can provide high-quality personnel training in the future.

Therefore, our research object was to examine the achievements of undergraduates enrolled in the 5-year program vs the achievements of students in the 4-year program. Through analysis of the achievements of the students in those programs, we understood the mastery of knowledge and professional skills among the students, which provided us with

Abbreviations

ANOVA, analysis of variance.

Key Laboratory of Tropical Translational Medicine, Ministry of Education, and School of Tropical Medicine and Laboratory Medicine, Hainan Medical University, Haikou, China

[#]These authors contribute equally to this work.
*To whom correspondence should be addressed.
ethan_ya@163.com

an intuitive basis for optimizing teaching methods, improving the quality of instruction, and reforming teaching methods.[3] We expect our research findings to be beneficial to the development of medical laboratory technology education.

Materials and Methods

Subject Individuals

We analyzed the achievements of 279 undergraduates from the first and most recent 2 graduating classes—students who had been enrolled in the 5-year system. For comparison, we also examined the achievements of the 252 undergraduates from the most recent 3 classes who will graduate after completing the 4-year system.

Evaluation of Student Achievements

Student achievements were calculated as follows: 40% of this value was comprised of the scores attained during the regular school year (herein referred to as the *usual scores*) and 60% was comprised of the scores in final examinations. The usual scores included attendance and quiz data. The questions for the final examinations came from standardized question banks, which contain more than 1000 questions for each course. Question types included multiple choice questions (single best answer multiple choice questions and multiple answer multiple choice questions), judgment questions, fill-in-the-blank questions, proper noun explanatory questions, case analysis questions, short answer questions, and discussion questions. The total scores were 100 points; the scoring method was called *centesimal grading*.

To assess achievements in a more brief fashion, we classified 100 marked scores into 5 levels. Scores of 90 and higher were classified as excellent, 80 to 89 as good, 70 to 79 as medium, 60 to 69 as passing, and less than 60 as failing. This scoring method was called *5-level form*.[4,5]

Undergraduates each defended their thesis before graduation. The graduation thesis was an experimental paper related to the medical laboratory. The achievements were scored comprehensively by 3 experts serving as judges.

Statistical Analysis

We used SPSS software, version 19.0 (IBM Corporation) was used for statistical analysis. The results were significant difference if the *P* value was less than .05. In centesimal grade, the average scores of all courses and those of all the same courses were compared separately, according to different year system and different course categories. Independent sample T-testing compared the average scores of different course categories such as general-education courses, professional basic courses, and professional courses, respectively, in the 5-year system and the 4-year system. We used analysis of variance (ANOVA) to compare the average scores of all courses in different year systems and those of all the same courses in different year systems.

We divided the grades into 5 levels: excellent, good, medium, pass, and fail. Within this categorization, we calculated the frequency in which student grades were within each level. We used χ^2 testing to compare the frequency of student grades being within each level for general-education courses, professional basic courses, and professional courses, comparing between the 5-year and 4-year programs.

Results

Curriculum Composition

Undergraduates in the medical-laboratory-technology program were required to complete 50 courses, which were divided into the following 3 categories: general-education courses, professional basic courses, and professional courses. Compared with the 5-year system, 4 courses were eliminated and 4 new courses added to the 4-year program (**Table 1**).

A lesson hour was 40 minutes long. There were 10 terms in the 5-year system, consisting of 8 terms in school (20 weeks per term; 160 weeks in total) and 2 terms of clinical practice in the hospital (a full year: 52 weeks). The 8 terms in school included 151 weeks for learning and 9 weeks for other activities, such as military training and short-term social practice. The number of credits for courses in the 5-year group was 220.5 in total, and the total education time was 3471 lesson hours. The average weekly education time was 22.99 lesson hours per week (3471 lesson hours / 151 weeks) in school. Learning in school accounted for 71.23% (151 weeks / [160 total weeks + 52 weeks in the year]) of the total weeks of the program. Clinical practice in the hospital accounted for 24.53% (52 / [160 + 52] of the total weeks of the program.

Table 1. Credits and Lesson Hours of Courses in the 5-Year and 4-Year Programs of Undergraduate Medical Laboratory Technicians in Hainan Medical University, China

Course Type	No.	Course Name	5-Year System		4-Year System	
			Credit(s)	Lesson Hours	Credit(s)	Lesson Hours
General Education	0	Advanced English	4	52	2	36
		Basic Computer Science	9	124	3	52
	2	College English	16	260	16	260
	4	Health Law	1.5	24	1.5	24
	5	Ideological, Moral, and Legal Education	3	48	3	48
	6	Information Retrieval and Utilization	2	40	2	40
	7	Mao Ze Dong System of Thought and Introduction to the Theoretical System of Socialism with Chinese Characteristics	6	96	5	80
	8	Medical Ethics	1.5	27	-	-
	9	Medical Higher Mathematics	3	45	3	48
	10	Mental Health Education	2	24	2	24
	11	Medical Psychology	4	60	2	36
	12	Military Science	-	-	2	30
	13	Occupation Development and Employment Guidance	2	35	2	35
	14	Outline of Modern Chinese History	2	32	2	32
	15	Physical Education	8	142	8	142
	16	Principle of Marxist Doctrine	3	48	3	48
	17	Situations and Policy	6	96	6	96
Professional Basic	18	Analytical Chemistry	4.5	72	4.5	72
	19	Biochemistry	4	64	4	64
	20	Epidemiology	2	36	2	36
	21	Experimental Chemical Analysis	2	33	2	33
	22	Histology and Embryology	3	48	3	48
	23	Inorganic Chemistry	6	82	6	82
	24	Instrumental Analysis	5	50	5	80
	25	Introduction to Clinical Medicine	10	160	7.5	120
	26	Medical Genetics	-	-	2	32
	27	Medical Immunology	5	75	3.5	54
	28	Medical Informatics	-	-	3	36
	29	Medical Physics	5	66	3	54
	30	Medical Statistics	2.5	40	2.5	40
	31	Organic Chemistry	5	82	5	80
	32	Pathogenic Biology	8	125	5	75
	33	Pathology	4	60	4	60
	34	Pathophysiology	2.5	38	2.5	38
	35	Physiology	4.5	70	5	70
	36	Systematic Anatomy	4	70	3	48
Professional	37	Advances in Clinical Immunology (Lectures)	1.5	24	1.5	24
	38	Advances in Clinical Microbiology (Lectures)	1.5	24	-	-
	39	Analysis of Clinical Laboratory Medical Records	2	32	-	-
	40	Basic Technology of Laboratory Medicine	7	143	7	143
	41	Diagnostics	6	100	-	-
	42	Exfoliative Cytology	-	-	1	19
	43	Introduction to Laboratory Medicine	3	36	3	36
	44	Laboratory Management	2	30	2	30
	45	Professional English	2	30	2	36
	46	Technology of Clinical Biochemistry	7	130	7	130
	47	Technology of Clinical Hematological Laboratory	7	108	7	126
	48	Technology of Clinical Immunology Examination	6	98	6	102
	49	Technology of Clinical Laboratory and Instruments	4	60	3	48
	50	Technology of Clinical Microbiology Examination	7.5	120	7	110
	51	Technology of Clinical Transfusion and Inspection	2	32	2	32
	52	Technology of Molecular Biology Diagnosis	6	94	5	90
	53	Technology of Parasitological Examination	4	54	4	54
	54	Tropical Medicine and Laboratory Medicine	2	32	2	32
Total			**220.5**	**3471**	**194.5**	**3165**

-, There was no course offered for students.

Lab Medicine 2021;52;93–101
DOI: 10.1093/labmed/lmaa033

There were 8 terms in the 4-year system, consisting of 6 terms in school (20 weeks per term; 120 weeks in total) and 2 terms of clinical practice in the hospital (44 weeks). The 6 terms in school included 117 weeks for learning and 3 weeks for other activities, such as military training and short-term social practice. The number of credits for courses in the 4-year group was 194.5 in total, and the education time was 3165 lesson-hours. The average weekly education time was 27.05 lesson-hours per week in school (3165 hours / 117 weeks). Learning in school accounted for 71.3% (117 / [120 + 44]) of the total program time. Clinical practice in the hospital accounted for 26.8% (44 / [120 + 44]) of the total program time.

Average Scores

The average (SD) score of the 5-year-program undergraduates in all courses was 77.94 (9.70), and that of the 4-year-program undergraduates was 76.35 (9.27) (F = 153.62; *P* <.001). The average score of the 5-year-program undergraduates in general-education courses was 79.28 (9.43), and that of 4-year undergraduates was 78.40 (8.60) (*t* = 4.83, *P* <.001). The average score of 5-year-program undergraduates in professional basic courses was 76.96 (9.57), and that of the 4-year-program undergraduates was 73.60 (8.67) (*t* = 14.72; *P* <.001). The average score of 5-year-program undergraduates in professional courses was 76.50 (10.09), and that of 4-year-program undergraduates was 75.66 (10.23) (*t* = 2.84; *P* = .005).

The average scores of 4-year-program undergraduates in general education courses, professional basic courses, professional courses, and all courses were significantly lower than those of 5-year-program undergraduates (*P* <.001; **Figure 1**). In the same courses, the average (SD) score of 5-year-program undergraduates was 78.04 (9.65), and that of 4-year-program undergraduates was 75.98 (9.16) (F = 261.48; *P* <.001).

The average (SD) score of 5-year-program undergraduates in the same general education courses was 79.22 (9.46), while that of 4-year-program undergraduates was 78.46 (8.60) (*t* = 4.14; *P* <.001). The average score of 5-year-program undergraduates in the same professional basic courses was 76.96 (9.57), and that of 4-year-program undergraduates was 72.61 (8.38) (*t* = 18.84; *P* <.001). The average score of 5-year-program undergraduates in the same professional courses was 77.02 (9.91), and that of

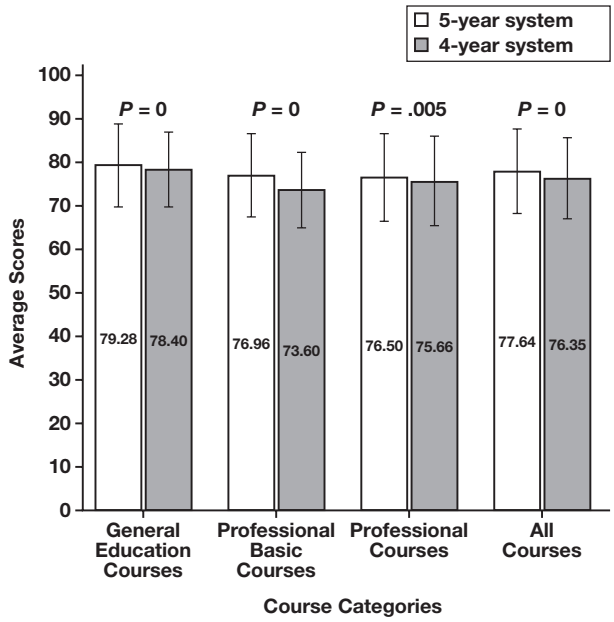


Figure 1

Average scores in different year systems were compared. Bars showed that the average scores, standard deviations and *P*-values of general-education courses, professional basic courses, professional courses and all courses of 5-year and 4-year programs.

4-year-program undergraduates was 74.86 (9.64) (*t* = 7.35; *P* <.001). The average scores of 4-year-program undergraduates in the same general-education courses, the same professional basic courses, the same professional courses, and all the same courses were significantly lower than those of the 5-year-program undergraduates, respectively (*P* <.001; **Figure 2**).

The average (SD) score of 5-year-program undergraduates for the graduation thesis was 80.91 (6.29). However, that of 4-year-program undergraduates was 76.69 (6.18), which was significantly lower than that of the 5-year-program students (*t* = 7.82; *P* <.001).

Scores Distribution

In all courses, the excellent score rate and the good rate in 4-year-program undergraduates were significantly lower than those in 5-year-program undergraduates (χ^2 = 123.18 [*P* <.001] in the excellent rate, and χ^2 = 14.85 [*P* <.001] in the good rate of the different-length programs). The medium rate (χ^2 = 28.61; *P* <.001), the passing rate (χ^2 = 31.97; *P* <.001), and the failure rate (χ^2 = 4.84; *P* = 0.03) were

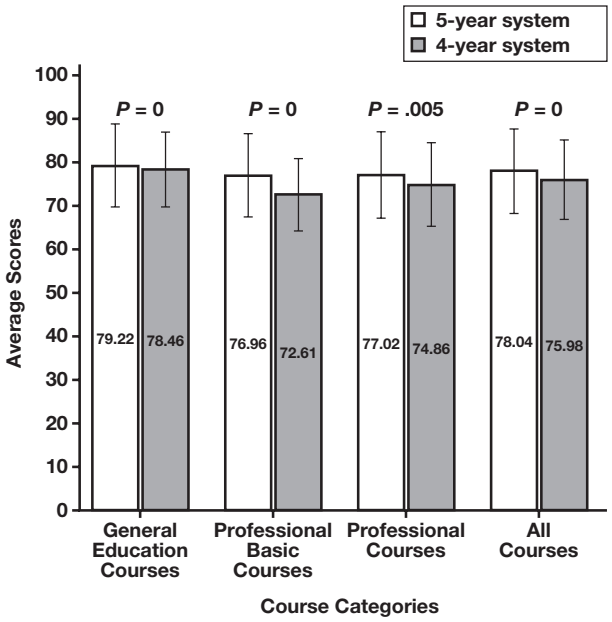


Figure 2

In common courses of different year systems, average scores were compared. Bars showed that the average scores, standard deviations and *P*-values of general-education courses, professional basic courses, professional courses, and all the same courses of 5-year and 4-year programs.

significantly higher than those in students in the 5-year program.

In general education courses, the excellent rate and the good rate in 4-year-program undergraduates were significantly lower than those among students in the 5-year program (χ^2 = 41.43 [*P* <.001] in the excellent rate of the different length programs, and χ^2 = 4.83 [*P* = 0.03] in the good rate of the different length programs). In contrast, the medium rate in the 4-year-program students was significantly higher than that in the 5-year-program students (χ^2 = 142.15; *P* <.001). There was no significant difference in the passing rate of students in the 5-year program and students in the 4-year program (χ^2 = 3.40; *P* = .06), as well as no significant difference in the failure rate between those groups (χ^2 = 0.56; *P* = .45).

In professional basic courses, the excellent rate in students in the 4-year program was significantly lower than that students in the 5-year program (χ^2 = 113.22; *P* <.001), and the passing rate was significantly higher than that in students in the 5-year program (χ^2 = 37.40; *P* <.001). There were no significant differences in the good rate (χ^2 = 0.74; *P* = .39), the medium rate (χ^2 = 0.115; *P* = .73), or the failure rate (χ^2 = 0.91; *P* = .34) between the 5-year-program and 4-year-program students.

In professional courses, the excellent rate in students in the 4-year program was significantly lower than that in students in the 5-year program (χ^2 = 4.413; *P* = .04), and the failure rate was significantly higher in students in the 4-year than in the 5-year program (χ^2 = 6.09; *P* = .01). There were no significant differences in the good rate (χ^2 = 3.43; *P* = .06), in the medium rate (χ^2 = 2.84; *P* = .09), and in the passing rate (χ^2 = 1.44; *P* = .23) between students in the 5-year and 4-year programs, respectively (**Figure 3**).

In all the same courses, the excellent rate (χ^2 = 106.23; *P* <.001) and the good rate (χ^2 = 34.70; *P* <.001) in students in the 4-year program were significantly lower than those of students in the 5-year program; the medium rate (χ^2 = 40.43; *P* <.001), the passing rate (χ^2 = 66.93; *P* <.001), and the failure rate (χ^2 = 5.10; *P* = .02) were significantly higher than that in the 5-year program. In the same general-education courses, the excellent rate of students in the 4-year program was significantly lower than that of students in the 5-year program (χ^2 = 30.51; *P* <.001), and the medium rate (χ^2 = 78.25; *P* <.001) was significantly higher than that in students of the 5-year program. There were no significant differences in the good rate (χ^2 = 1.62; *P* = .20), the passing rate (χ^2 = 1.73; *P* = .19) and the failure rate (χ^2 = 0.608; *P* = .44).

In the same professional basic courses, the excellent rate (χ^2 = 111.80; *P* <.001) and the good rate (χ^2 = 19.88; *P* <.001) of students in the 4-year program was significantly lower than those of students in the 5-year program; the passing rate of 4-year-program students was significantly higher than that of 5-year-program students (χ^2 = 73.48; *P* <.001). There were no significant differences in the medium rate (χ^2 = 1.86; *P* = .17) and in the failure rate (χ^2 = 0.74; *P* = .39) between students in the 5-year and the 4-year programs, respectively.

In the same professional courses, the excellent rate (χ^2 = 7.06; *P* = .008) and the good rate (χ^2 = 6.43; *P* = .01) of 4-year-program students were significantly lower than those in 5-year-program students; the passing rate (χ^2 = 8.91; *P* = .003) and the failure rate (χ^2 = 5.20; *P* = .02) were significantly higher than those among students in the 5-year program. There was no significant difference in the medium rate between student groups (χ^2 = 1.13; *P* = .29; **Figure 4**).

DOI: 10.1093/labmed/lmaa033

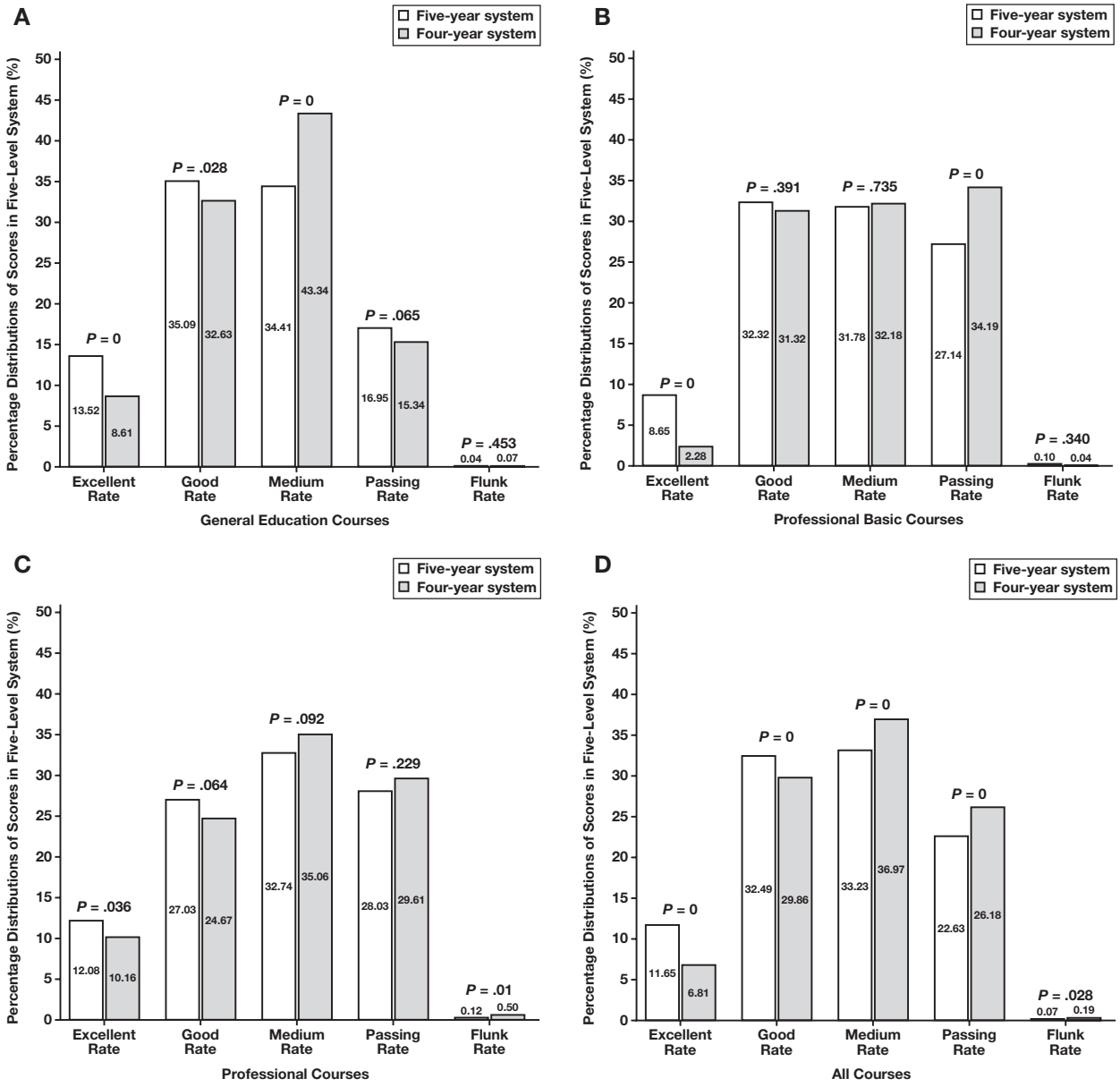


Figure 3

Percentage distributions of scores in 5 levels were compared. Bars showed that percentages and *P*-values of general-education courses (**A**), professional basic courses (**B**), professional courses (**C**) and all courses (**D**) in 5-year and 4-year programs.

In the graduation thesis category, the good rate of students in the 4-year program was significantly lower than that of students in the 5-year program ($\chi^2 = 3.73$; $P > .049$); the medium rate ($\chi^2 = 40.25$; $P < .049$) and the passing rate ($\chi^2 = 40.43$; $P < .001$) were significantly higher than those of students in the 5-year program. There was no significant difference in the excellent rate between the student groups ($\chi^2 = 3.73$; $P > .049$).

Discussion

The cultivation of medical laboratory technicians requires undergraduates to have strong humanistic qualities[6]; to master the theoretical knowledge and practical skills of basic medicine, clinical medicine, and medical laboratory science; to perform hospital inspection tasks skillfully and

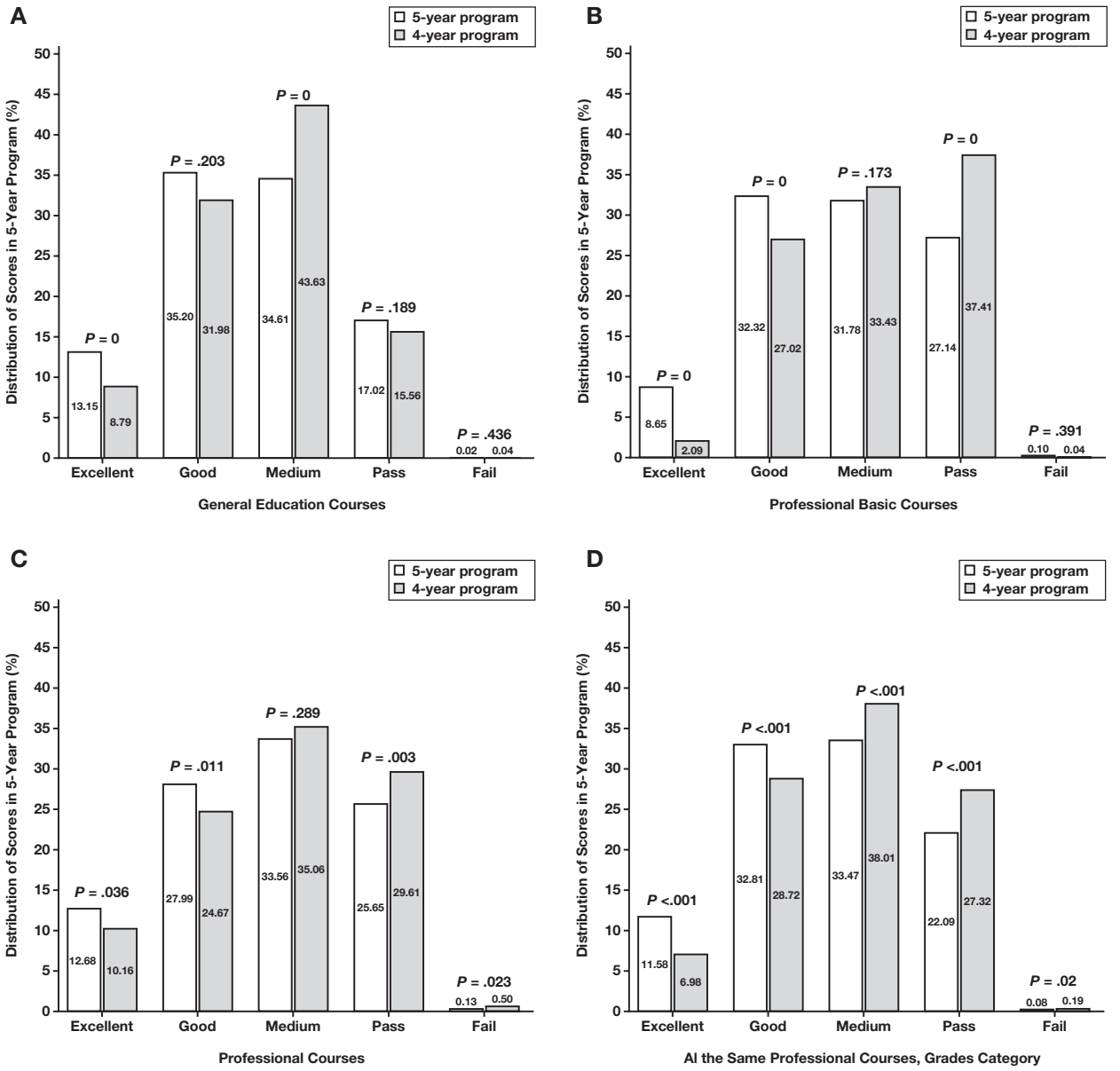


Figure 4

In common courses of different year systems, percentage distributions of scores in 5 levels were compared. Bars showed that percentages and *P*-values of general-education courses (**A**), professional basic courses (**B**), professional courses (**C**) and all the same courses (**D**) in 5-year and 4-year programs.

accurately; to understand the performance,[7] working principles, and operational technology of common inspection instruments; to be familiar with basic national healthcare policies; to know the rules and regulations of clinical laboratory management well; and to comprehend trends in medical laboratory technology and the development of emerging disciplines.[8,9] Analysis of curriculum achievements can directly reflect the knowledge and skills of undergraduates while they are in school. By comparing the average scores in general education courses, professional basic courses, and professional courses, we discovered the average scores of the 4-year-program undergraduates

Lab Medicine 2021;52;93–101
DOI: 10.1093/labmed/lmaa033

in all courses were significantly lower than those scores in 5-year-program undergraduates.

These data reflect a situation in healthcare training that is not particularly optimistic. The shorter number of training-years has made learning tasks more onerous for students.[10] The goal of the new 4-year program is to train students to apply their talents, with the aim of increasing the number of skilled medical laboratory practitioners working in hospitals, to help meet the needs of the larger society. Teachers and undergraduates in China are being required to adapt to the new year-system as soon as possible.[11]

General education refers to "nonprofessional education" for all university undergraduates, aimed at cultivating well-rounded individuals and citizens.[12,13] The idea of general education in universities should be to cultivate high-level, civilized, complete, humanistic education for learners, with broad vision, comprehensive understanding, broad-minded spirit, and strong emotional foundations.[14] It does not directly prepare learners for the application of their knowledge or help them to develop certain abilities. It covers a wide range of comprehensive subjects and is concerned with human life, morals, and emotional and rational harmonious development.[15]

Professional basic courses are set up to lay a necessary foundation for the study of the subjects covered in professional courses. Completion of these basic courses becomes crucial for undergraduates as they strive to master further areas of professional knowledge.

Professional courses refers to courses that impart professional knowledge and skills.[16,17] The task of professional courses is to enable undergraduates to master necessary professional theories; the training goal is to become a qualified medical laboratory technician.[18] Students should be trained to keep abreast of emerging science, technology, and development trends, and learn to analyze and solve practical problems.

Medical laboratory science is a highly specialized discipline. Hence, undergraduates majoring in medical-laboratory technology should have solid theoretical knowledge and practical skills, a strong sense of medical safety procedures, a preliminary ability to evaluate various laboratory results, and the ability to manage and control laboratory work and quality.[19,20] They should develop a certain sense of innovation and scientific research ability; they should also be able to effectively handle interpersonal communication. In the workplace, they must pay attention to teamwork. They should find the answers to work-based and learning problems in a timely manner, while developing their ability to solve problems independently.[21] These qualities are useful when pursuing continuing education after graduation, and form the basis for lifelong further development and improvement.

Conclusions

This study was an unprecedented (to our knowledge) assessment of the training quality of medical laboratory undergraduate programs with lengths of different numbers of school years. The transformation from the 5-year undergraduate training program in China to a new 4-year program is an important reform in medical laboratory education.[22,23] It reflects the plan of the Ministry of Education of Hainan Province, China, for the orientation of the medical laboratory specialty, which is built on medical knowledge and focuses on the cultivation of technology skills.

We in China should take measures that will improve the achievement levels and practical skills of 4-year-program undergraduates, as they strive to adapt to the new training plan in adequate time. In doing so, as educators, we reinforce the importance of teaching standards and of improving the comprehensive skill sets of our undergraduate students.[24] Doing so will provide effective measures to optimize the structural knowledge obtained by undergraduates and to improve the professional, practical skills of teachers in training modern medical-laboratory technicians. **LM**

Funding Sources

This study was supported by grants from the Education Research Project of Hainan Medical University, Haikou, China (HYP201928) and the Education Department of Hainan Province (Hnjg2019ZD-18, Hnjg2020ZD-33).

Acknowledgments

We thank Hainan Medical University, Haikou, China, and The Education Department of Hainan Province, China, for

their financial support of our research. YL analyzed the data and wrote the manuscript. QX designed the study and collected the data. JY performed revisions to the draft report and approval of final manuscript. All authors read and approved the final manuscript.

References

1. Arnold L, Cuddy PG, Hathaway SB, Quaintance JL, Kanter SL. Medical school factors that prepare students to become leaders in medicine. *Acad Med.* 2018;93(2):274–282.
2. Powell DE, Carraccio C. Toward competency-based medical education. *N Engl J Med.* 2018;378(1):3–5.
3. Gonzalo JD, Dekhtyar M, Starr SR, et al. Health systems science curricula in undergraduate medical education: identifying and defining a potential curricular framework. *Acad Med.* 2017;92(1):123–131.
4. Sinclair HK, Cleland JA. Undergraduate medical students: who seeks formative feedback? *Med Educ.* 2007;41(6):580–582.
5. Tootoonchi M, Yamani N, Changiz T, Yousefy A. Research priorities in medical education: a national study. *J Res Med Sci.* 2012;17(1): 83–91.
6. Dymond JS, Scheifele LZ, Richardson S, et al. Teaching synthetic biology, bioinformatics and engineering to undergraduates: the interdisciplinary Build-a-Genome course. *Genetics.* 2009;181(1): 13–21.
7. Dehghani M, Athar O, Ashourioun V, et al. A transitional curriculum for preparing medical students for internship, does it work? *J Res Med Sci.* 2013;18(6):506–509.
8. Igbokwe UL, Nwokenna EN, Eseadi C, et al. Intervention for burnout among English education undergraduates: implications for curriculum innovation. *Medicine (Baltimore).* 2019;98(26):e16219.
9. Sundberg MD, Moncada GJ. Creating effective investigative laboratories for undergraduates. *Bioscience.* 1994;44(10):698–509.
10. Caverzagie K, Lane S, Donnelly J, et al. Proposed performance-based metrics for the future funding of graduate medical education: starting the conversation. *Acad Med.* 2017;93(7):1002–1013.
11. Olupeliyawa A, Balasooriya C. The impact of programmatic assessment on student learning: what can the students tell us? *Med Educ.* 2015;49(5):453–456.
12. Dacre JE, Fox RA. How should we be teaching our undergraduates? *Ann Rheum Dis.* 2000;59(9):662–667.
13. Iversen AC, Eady NA, Wessely SC. The role of mentoring in academic career progression: a cross-sectional survey of the Academy of Medical Sciences mentoring scheme. *J R Soc Med.* 2014;107(8):308–317.
14. Fasel JHD. Teaching of gross anatomy to medical undergraduates: general practice as a guideline? A synopsis. *J Anat.* 2002;192(2):305–306.
15. Engbers R, Fluit CR, Bolhuis S, Sluiter R, Stuyt PM, Laan RF. Relations between policy for medical teaching and basic need satisfaction in teaching. *Adv Health Sci Educ Theory Pract.* 2015;20(4):969–980.
16. Hoy RJ. Radiology in medical undergraduate education. *Australas Radiol.* 1974;18(3):269–274.
17. Dornan TL, Grenfell A, Heller SR, Morrish N, Tooke J. Diabetes in the undergraduate medical curriculum: a response to 'Tomorrow's' doctors'. *Diabet Med.* 1997;14(4):275–278.
18. Brosin HW. Undergraduate medical education. *West J Med.* 1990;17(4):609–616.
19. Jones GL, Walley T, Bligh J. Integrating clinical pharmacology in a new problem based medical undergraduate curriculum. *Br J Clin Pharmacol.* 1997;43(1):15–19.
20. Kumar R, Dhaliwal U. Career choices of undergraduate medical students. *Natl Med J India.* 2011;24(3):166–169.
21. Havemann M, Bösner S. Global Health as "umbrella term" - a qualitative study among Global Health teachers in German medical education. *Global Health.* 2018;14(1):32.
22. Griffin B. Selecting medical students: considering qualities other than academic ability. *Med Educ.* 2018;52(1):9–11.
23. Yanes AF. The culture of perfection: a barrier to medical student wellness and development. *Acad Med.* 2017;92(7):900–901.
24. Gonzalo JD, Caverzagie KJ, Hawkins RE, Lawson L, Wolpaw DR, Chang A. Concerns and responses for integrating health systems science into medical education. *Acad Med.* 2017(6):843–849.

DOI: 10.1093/labmed/lmaa033

Case Study

A Rare Cohort of Two Rh_{null} Individuals

Richard R. Gammon, MD,[1] Alexander Delk, MT(ASCP)SBB[CM],[2] Patricia Houtz, BS, MT(ASCP),[3] Harold Alvarez, MD,[4] Nancy Benitez, BS, MHS(ASCP)SBB[CM1]*

Laboratory Medicine 2021;52:e1-e4

DOI: 10.1093/labmed/lmaa009

ABSTRACT

Objective: A 77 year old female was admitted with a subdural hematoma requiring 1 unit of apheresis platelets. She was a study subject in the 1960s and was found to be Rh_{null}, along with another individual who previously served as a directed donor for her.

Methods: Serologic testing performed by the immunohematology reference laboratory (IRL) confirmed that the patient was Rh_{null} and expressed anti-Rh29 antibodies. While searching for red blood cells (RBCs) for possible transfusion, it was discovered that the individual from the original study had recently donated an autologous unit.

Results: The IRL discovered that the donor's antigen typing was r'r'. Testing had been performed using a molecular human erythrocyte antigen BeadChip (HBC). Due to the discrepancy between current and historical testing results, a donor segment was thawed and by tube testing confirmed to be Rh_{null}. A limitation of HBC is that many null phenotypes will be missed.

Conclusion: This case demonstrated that Rh_{null} evaluation of the donor required both serological and molecular methods.

Keywords: antibodies to high prevalence antigens, high prevalence, Rh blood group system, null types, complex antibody identification, platelet transfusion, antigen testing, antigen testing by DNA, *RHAG*

A 77 year old female was admitted to the emergency room (ER) with a subdural hematoma that occurred as the result of a fall. To reduce the risk of additional bleeding, an order was placed for 1 unit of apheresis platelets. The physician's assistant called the transfusion service stating that the patient had a history of being Rh_{null}. As the hemoglobin and hematocrit levels had decreased within the first 6 hours of admission (**Figure 1**) and there was a concern that the patient may need a red blood cell (RBC) transfusion, the hospital blood bank supervisor (HBBS) called the ER and was transferred to the husband of the patient. He had several papers that had been published in 1967 reporting the patient's history of being Rh_{null}.[1,2] He confirmed that the patient had a Rh_{null} friend whom she met and was also part of the 1967 investigation. The friend had previously donated for the patient and now lived in the area. The patient's husband provided contact information.

The HBBS contacted the friend who stated that she had within the last year donated an autologous unit that was being stored at the local blood center. The friend was not able to provide a directed donation at this time since she had surgery scheduled.

The Rh_{null} phenotype is rare, with a lack of expression of all Rh antigens. It is associated with stomatocytosis, spherocytosis, increased osmotic fragility, and elevated Na^+/K^+ ATPase activity. The current Food and Drug Administration-approved molecular testing may not be able to detect Rh_{null}, requiring serological testing to confirm its presence.

Abbreviations:

IRL, immunohematology reference laboratory; RBCs, red blood cells; HBC, human erythrocyte antigen BeadChip; ER, emergency room; HBBS, hospital blood bank supervisor; SPRCA, solid-phase red cell adherence; DAT, direct antiglobulin testing; PEG, polyethylene glycol.

[1]Immunohematology Reference Laboratory-Cypress Creek, OneBlood, Inc., Ft. Lauderdale, Florida, [2]Scientific Medical and Technical Administration, OneBlood, Inc., Orlando, Florida, [3]Jupiter, Florida, [4]Miami Cancer Institute, Miami, Florida

*To whom correspondence should be addressed.
richard.gammon@oneblood.org

Materials and Methods

A type and screen and 1 unit of apheresis platelets were ordered by the hospital ER physician. The hospital performed the solid-phase red cell adherence (SPRCA) antibody screen and subsequent panel.

As part of the protocol for possible RBC transfusion, the patient's specimen was sent to the immunohematology reference laboratory (IRL) for confirmation and additional antibody exclusion.

The IRL performed ABO, Rh, and direct antiglobulin testing (DAT), a polyethylene glycol (PEG) screen, autocontrol test, and RBC antigen phenotyping (CcEe, K, Fy^aFy^b, Jk^aJk^b, MNSs). The patient's history of $Rh_{null,}$ typing confirmed by the IRL, as well as the frequency and the strength of the antibody reactivity, led to the performance of 4 allogeneic adsorptions with cells selected based upon the patient's phenotype to remove the high-incidence Rh antibody. An eluate was prepared from the cells used for adsorption to help identify high-incidence antibodies. An aliquot of the specimen was sent to the blood center's molecular laboratory for additional testing.

Results

The patient was found to be *Asub* negative with anti-A1 and a negative DAT result. The RBC antigen profile was determined by serology (**Table 1**). The plasma reacted 4+ with an antibody screen and panel at SPRCA and PEG anti-human globulin. The autocontrol was negative. Alloantibodies were not detected in the adsorbed plasma. Since R1R1 cells were used to adsorb the plasma, anti-D, -C, -e were not excluded; however, upon the completion of adsorption, the IRL was notified by the HBBS of the history of anti-Rh29. Since anti-Rh29 reacts with any cells that express Rh antigens, it was not necessary to rule out anti-D, -C, -e since blood for transfusion would need to be negative for all Rh antigens. A peripheral blood smear revealed the presence of stomatocytes (**Figure 2**).

The patient's Rh_{null} friend had recently donated an autologous unit that was frozen. At the time of donation, the unit was tested by a molecular human erythrocyte antigen BeadChip (HBC) (BioArray Solutions, Ltd., Warren, NJ), and the predicted RHCE phenotype was (C+E-c-e+). This result together with the donor screening serological ABO RhD result predicted that the donor was A negative r'r' (dCe/dCe). Since this result did not match the donor's history of being Rh_{null}, the IRL tested cells from the donated unit by serological methods, and they were found to be Rh_{null}, consistent with the 1967 study results.[2] The

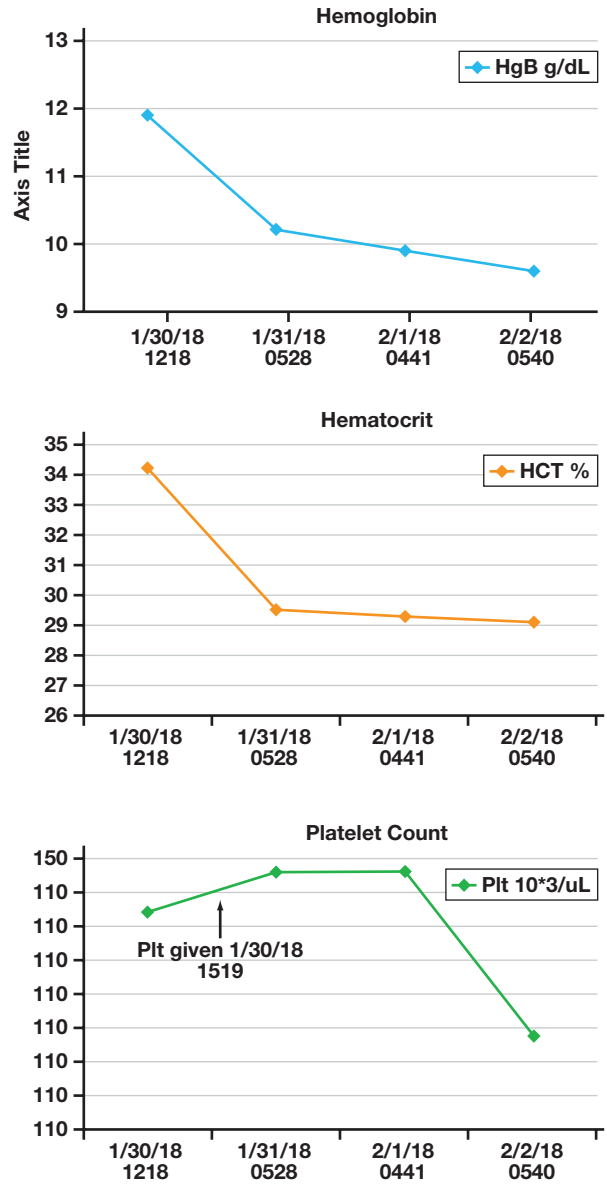


Figure 1

There was a drop in the hemoglobin and hematocrit within six hours of admission that necessitated a search for compatible blood. The graphs also shows where the patient received an Apheresis Platelet transfusion with subsequent increase in platelet count.

molecular HBC package insert indicated that Rh_{null} may not be detected.[3]

An HBBS contacted the IRL to confirm that standard donor platelets could be issued to an Rh_{null} patient. Due to the very low RBC content of apheresis platelets ($\leq 0.4 \times 10^9$ RBCs–1.0×10^9 RBCs), it was decided to transfuse the

Table 1. Patient Antigen Profile

D	C	c	E	e	K	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
0	0	0	0	0	0	0	+	+	0	+	+	+	+

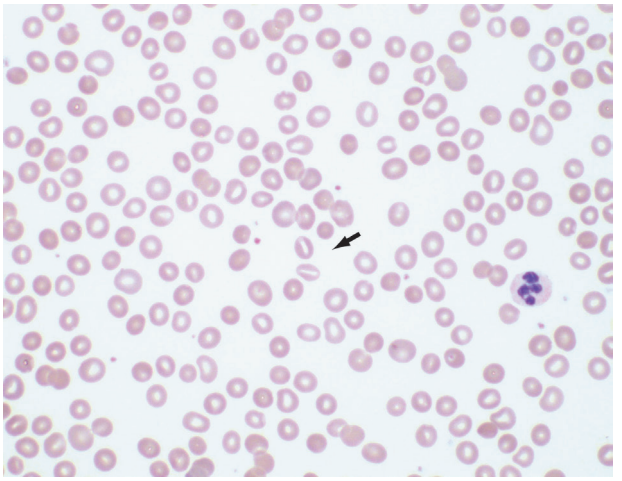


Figure 2

Peripheral blood smear from Rhnull patient demonstrating characteristic stomatocytes (arrow).

apheresis platelets from a non-Rh_{null} donor.[4,5] Neither posttransfusion hemolysis nor a transfusion reaction was noted. The patient did not require an RBC transfusion. She recovered and was discharged to home.

Discussion

The Rh_{null} phenotype is exceedingly rare, with an incidence of 1 in 6,000,000.[6] Erythrocytes express a third Rh protein, RhAG, which shares 38% of its identity with RhD/RhCE, has the same topology in the membrane, and is encoded by a single gene on chromosome 6.[7] RhAG associates with the Rh blood group proteins in the membrane to form an Rh-core complex. Four red cell antigens resulting from a single amino acid substitution form the RhAG blood group system.[7]

Red blood cells lacking all Rh antigens are designated Rh_{null}.[8] Although exceedingly rare, the phenotype most often results from nucleotide changes in *RHAG* known as a "regulator"-type Rh_{null}, indicating that the RHAG protein plays a critical role in trafficking RHCE and RHD to the membrane, as was seen with the donor in this case.[7] Less often, Rh_{null} individuals have *RHCE* nucleotide changes along with the common deletion of *RHD*, and these individuals are called amorphs.[7]

Common features of Rh_{null} RBCs are stomatocytes (**Figure 2**) and mild anemia, suggesting that the Rh proteins have an important structural role in the erythrocyte membrane.[7] Other findings include spherocytosis, increased osmotic fragility, altered phospholipid asymmetry, altered cell volume, defective cation fluxes, and elevated Na^+/K^+ATPase activity.[8–12] The Rh complex is associated with the membrane skeleton through CD47 protein 4.2, ankyrin band 3, Duffy, and glycophorin B and C interactions.[13,14]

Typical Rh antibodies do not activate complement.[6] As a result, in a transfusion reaction involving Rh antibodies, hemolysis is primarily extravascular rather than intravascular.[6] Although not relevant with this case due to the patient's age, Rh antibodies have the potential to cause clinically significant hemolytic disease of the fetus and newborn.[2] Antibodies to high-prevalence Rh antigens include anti-Rh29 made by some Rh_{null} individuals, including the patient in this case, who lacked Rh antigens.[6]

Rh_{null} RBCs have a shortened *in vivo* survival, and patients often demonstrate a compensated hemolytic anemia.[15,16] Transfusion of patients with anti-Rh29 is a concern because only the Rh_{null} RBCs will be compatible. Individuals with the Rh_{null} phenotype are not only exceedingly rare in general, but they are unlikely to meet standard predonation criteria as a result of anemia. In this particular case, the donor's hemoglobin level was 13.5 g/dL, and she would have met US Food and Drug Administration's minimum requirements for females of 12.5 g/dL for a directed or allogeneic donation; however, due to an upcoming surgery, this was not possible.[17]

Evaluation of Rh_{null} status requires both serological and molecular methods to ensure detection. As with the methodology used, the molecular HBC package insert indicated that Rh_{null} may not be detected.[3] Genetic testing capable of identifying underlying mutations, amorph or regulator type of *RHCE*/

DOI: 10.1093/labmed/lmaa009

RHD and *RHAG* genes, respectively, should be performed to correlate the serology. Good communication between the patient, hospital, and IRL is critical to ensure that appropriate testing is performed. This case demonstrated that due to the low number of RBCs in apheresis platelets, a non-Rh_{null} donor was successfully transfused without product modification (eg, washing) or without an adverse event (eg, hemolysis). The 2 Rh_{null} study subjects from the 1960s who met over 50 years prior eventually became friends, with 1 serving as a directed donor many years ago for the other during a pregnancy. **LM**

References

1. Haber GV, Bastani A, Arpin PD, et al. Rh_{null} and pregnancy complicated by maternal anti-"total Rh." I. Anti-Rh29. *Transfusion.* 1967;7:389.
2. Bar-Shany S, Bastani A, Cuttner J, et al. Rh_{null} and pregnancy complicated by maternal anti-"total Rh" II. Additional evidence of multiple anomalies associated with the Rh-29m (rh_m) variety of Rh_{null} red cells. *Transfusion.* 1967;7:389–390.
3. Seidl S, Spielmann W, Martin H. Two siblings with Rh_{null} disease. *Vox Sang.* 1972;23(3):182–189.
4. Berséus O, Högman CF, Johansson A. Simple method of improving the quality of platelet concentrates and the importance of production control. *Transfusion.* 1978;18(3):333–338.
5. Wagner SJ. Whole blood and apheresis collections for blood components intended for transfusion. In: Fung MK, *Technical Manual*. 19th ed. Bethesda, MD: AABB Press; 2017.
6. *PreciseType HEA Molecular BeadChip test. Package insert 190-20210-EN Rev D*. Immucor; 2018. https://qualtexlabs.org/sites/default/files/precisetype_hea_beadchip_test-ivd_packageinsert-190-20210_revb_003.pdf. Accessed October 31, 2019.
7. Denomme GA. Rh system. In: Fung MK, ed. *Technical Manual*. 19th ed. Bethesda, MD: AABB Press; 2017.
8. Agre P, Cartron JP. Molecular biology of the Rh antigens. *Blood.* 1991;78(3):551–563.
9. Anstee DJ, Tanner MJ. Biochemical aspects of the blood group Rh (rhesus) antigens. *Baillieres Clin Haematol.* 1993;6(2):401–422.
10. Ballas SK, Clark MR, Mohandas N, et al. Red cell membrane and cation deficiency in Rh_{null} syndrome. *Blood.* 1984;63(5):1046–1055.
11. Nash R, Shojania AM. Hematological aspect of Rh deficiency syndrome: a case report and a review of the literature. *Am J Hematol.* 1987;24(3):267–275.
12. Kuypers F, van Linde-Sibenius-Trip M, Roelofsen B, Tanner MJ, Anstee DJ, Op den Kamp JA. Rh_{null} human erythrocytes have an abnormal membrane phospholipid organization. *Biochem J.* 1984;221(3):931–934.
13. Dahl KN, Parthasarathy R, Westhoff CM, Layton DM, Discher DE. Protein 4.2 is critical to CD47-membrane skeleton attachment in human red cells. *Blood.* 2004;103(3):1131–1136.
14. Nicolas V, Le Van Kim C, Gane P, et al. Rh-RhAG/ankyrin-R, a new interaction site between the membrane bilayer and the red cell skeleton, is impaired by $Rh(_{null})$-associated mutation. *J Biol Chem.* 2003;278(28):25526–25533.
15. Avent ND, Reid ME. The Rh blood group system: a review. *Blood.* 2000;95(2):375–387.
16. Ooley P. *AABB Standards for Blood Banks and Transfusion Services*. 31st ed. Bethesda, MD: AABB Press; 2017.
17. US Department of Health and Human Services. Code of Federal Regulations 21 CFR 630.10 (f) (3). 2019. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=630.1. Accessed October 31, 2019.

Case Study

A Dozen Testosterone Samples From One Patient, on One Day?

*Kevin F. Foley, PhD**

Laboratory Medicine 2021;52:e5-e7

DOI: 10.1093/labmed/lmaa032

ABSTRACT

The differential diagnosis of female virilization and infertility can be significantly narrowed using routine laboratory testing. The case presented herein is an example of a 28 year old Caucasian female patient with amenorrhea, hirsutism, and infertility in the context of markedly elevated serum testosterone levels. This case highlights the use of bilateral ovarian vein sampling for testosterone as a means to localize the ectopic testosterone production and to guide future surgical procedures. Adrenal vein sampling procedures are relatively more common than other methods. Ovarian vein sampling is less common, yet in this case, it proved diagnostic. This case demonstrates the needed cooperation of the clinical laboratory and the patient care team performing the catheterization, for this type of testing to be useful. In this unique case, we discovered bilateral production of androgens.

Keywords: testosterone, tumor, ovarian, oophorectomy, sampling, cortisol

Patient History

A 28 year old Caucasian woman presented to her primary-care physician with the chief complaint of infertility. She explained that she had attempted to conceive for the past 7 years but had not been successful. On further questioning the patient reported she had not menstruated for the past 10 years. By querying the electronic medical record (EMR) for this patient, we discovered that 5 years previously, a serum total testosterone concentration of 134 ng per dL had been recorded. At that time, a diagnosis of polycystic ovarian syndrome (PCOS) had been made, and amenorrhea was also noted. No other endocrine testing had been performed since that time, and care was now being established with a new physician.

Abbreviations:

EMR, electronic medical record; PCOS, polycystic ovarian syndrome; CT, computed tomography; RR, reference range; DHEA-S, dehydroepiandrosterone sulfate; IVC, inferior vena cava; DHEA, dehydroepiandrosterone; CAH, congenital adrenal hyperplasia; … , nonapplicable.

Department of Pathology, Kaiser Permanente Northwest, Portland, Oregon

*To whom correspondence should be addressed.
Kevin.f.foley@kp.org

Taking a thorough medical history revealed that the patient had experienced increasing and significant virilization during the past 2 years. She had developed noticeable facial hair and marked androgenic-patterned hair loss. The patient was referred to an endocrinologist, who ordered hysterosalpingography to assess tubal patency in relation to the infertility chief complaint.

The hysterosalpingography revealed no abnormal findings. A pelvic ultrasound was performed, which revealed multiple small peripheral follicles in both ovaries, with an overall impression consistent with PCOS. Aside from the mention of PCOS, the pelvic ultrasound results were otherwise unremarkable. A computed tomography (CT) scan was conducted, which revealed a small radiographically-unremarkable adrenal nodule.

Clinical and Laboratory Information

Given the unremarkable imaging findings stated in the previous paragraph, the endocrinologist ordered laboratory

testing to investigate the virilization. The total testosterone level recorded was 314 ng per dL. This concentration is within the normal range for males but abnormally elevated for females (female reference range [RR], 2–45 ng/dL). The free testosterone level for the patient was 38.9 pg per mL (RR, 0.1–6.4 pg/mL).

Given the significant degree of virilization and the elevated testosterone levels, the endocrinologist treating the patient suspected an adrenal adenoma, which might be evidenced in the finding of the unremarkable but visible adrenal nodule. Dehydroepiandrosterone sulfate (DHEA-S), 17-hydroxyprogesterone, and 24-hour urinary free cortisol testing was ordered to help assess the source of the excess testosterone. Laboratory testing revealed that DHEA-S, 17-hydroxyprogesterone, and urinary free cortisol were within normal adult female ranges (DHEA-S, 90 μg/dL [RR, 18–391 μg/dL]; 17-hydroxyprogesterone, 61.18 ng/dL [RR, < 206.00 ng/dL]; urine free cortisol, 29.0 μg/g creatinine [RR, 3.1–42.3 μg/g creatinine]).

DHEA-S testing results in this patient were unremarkable. The results of these laboratory tests suggest that an adenoma of ovarian origin was a more likely cause for the ectopic testosterone secretion than an adrenal adenoma.

With this information, a bilateral adrenal and ovarian vein catheterization procedure was coordinated with the Department of Radiology and the laboratory. The intent of the procedure was to identify the possible presence of a unilateral, testosterone-producing adenoma and to localize the possible adenoma as being ovarian or adrenal.

The radiologist performing the procedure successfully catheterized the right and left adrenal veins, as well as the right and left ovarian veins. Plasma specimens were collected from the relative catheters, and specimens were carefully labeled with the correct anatomic location. Serial measurements were taken to ensure specimen and assay precision. Also, peripheral cortisol measurements were collected, along with the vein-catheterized specimens. The cortisol specimens helped confirm the correct placement of catheters because cortisol concentrations should be higher from adrenal veins than from peripheral veins. The results from the procedure are shown in **Table 1**.

Discussion

The significant increase in virilization during the past 2 years, rather than a more gradual progression that commenced with the diagnosis of PCOS, suggested that the symptoms experienced by the patient may not be entirely attributable to PCOS. In the context of a female patient with elevated testosterone, infertility, virilization, and possible ovarian or adrenal mass, the differential diagnosis is fairly narrow and includes ovarian neoplasm, ovarian hyperthecosis, and adrenal mass. In our experience, a unilateral ovarian neoplasm is the most common finding in such cases.

This patient had a pre-existing diagnosis of PCOS. However, although PCOS is associated with infertility and elevated testosterone levels, her continued virilization, coupled with her persistent and highly elevated testosterone level, suggested that another pathologic manifestation was present. Given that CT imaging had only shown a small, radiographically unremarkable adrenal nodule and that pelvic ultrasound failed to show significant ovarian masses, we decided that a bilateral catheterization procedure could help clarify the source of the ectopic testosterone.

During the catheterization, whole blood specimens from the bilateral adrenal and ovarian veins were collected for testosterone measurements. Because very high concentrations of testosterone were expected, we reasoned that the analytical measurement range of our on-site laboratory was likely inadequate for specimen analysis. Therefore, specimens were sent to a reference laboratory that had validated a testosterone assay with an analytical measuring range that extended to 10,000 ng per dL. Whole blood from the bilateral adrenal veins and inferior vena cava of the patient were also collected for cortisol measurements. Vena cava measurements served as peripheral controls, to contrast with the results from the adrenal veins and thus confirm catheterization of the adrenal veins. The results are shown in **Table 1**.

The hospital laboratory received 21 specimens from this procedure. These specimens all needed to be sent to our main laboratory (located miles away from our hospital), and the anatomic draw locations needed to be added to the notes for each specimen order. Hence, it was important for the laboratory to be prepared for this procedure. A laboratory staff member was assigned to the

Table 1. Results of Bilateral Adrenal and Ovarian Vein Catheterization Procedure[a]

		Cortisol (µg/dL)		
Specimen No.	**Right Adrenal Vein**	**Left Adrenal Vein**	**Peripheral (IVC)**	
Specimen 1	12.6	7.4	2.4	…
Specimen 2	14.2	6.8	2.4	…
Specimen 3	8.8	7	2.4	…
Testosterone (ng/dL)				
Specimen No.	**Right Adrenal Vein**	**Left Adrenal Vein**	**Right Ovarian Vein**	**Left Ovarian Vein**
Specimen 1	194	157	>10,000	6097
Specimen 2	185	162	8117	5227
Specimen 3	192	176	5575	4606

Abbreviations: *IVC, inferior vena cava; … , nonapplicable.*
[a]*Patient is a 28 year old Caucasian woman.*

procedure to oversee specimen processing and maintain the integrity and location specificity of the specimens. Without laboratory involvement, transfer of the anatomic location notes for each specimen would not have crossed into the EMR, making interpretation of this intensive procedure impossible.

Testing results revealed that the right adrenal gland was producing more cortisol than the left. Correct catheterization was confirmed, with the finding that the peripheral (inferior vena cava [IVC]) cortisol level was lower than that in both adrenal veins. However, because the patient did not have Cushing disease, the asymmetrical cortisol concentrations were deemed to be clinically insignificant. Thus, cortisol served its role as a control, to assure correct placement of the adrenal vein catheters.

Dehydroepiandrosterone (DHEA) and DHEA-S, as well as androstenedione, are mainly produced in the adrenal glands. Thus, an increased DHEA-S concentration is used as a marker of increased adrenal activity.[1] Therefore, elevated DHEA levels are commonly associated with adrenal tumors.[2]

Adult onset congenital adrenal hyperplasia (CAH) in females can lead to hirsutism and infertility. For this reason, we measured 17-hydroxyprogesterone in our patient. However, her 17-hydroxyprogesterone levels were found to be within the normal range. Although not all cases of CAH present with elevated 17-hydroxyprogesterone,[3] our findings suggested that CAH was less likely. CAH due to steroid 21-hydroxylase gene (*CYP21A2*) mutations typically shows highly-elevated 17-hydroxyprogesterone levels. Adrenal tumors secreting only testosterone, with no concomitant increase of DHEA-S, are extremely rare.[4]

Because DHEA-S and 17-hydroxyprogesterone levels were normal in this patient, a testosterone-secreting ovarian adenoma seemed more likely than excess androgens from the adrenal glands. We hoped that the bilateral catheterization procedure would help discriminate the findings further.[5] The procedure revealed that testosterone, the hormone of interest, was significantly elevated in the right ovary, compared with the left ovary. However, both ovaries secreted significantly high levels of testosterone.

Because the patient was eager to conceive, we hoped we would discover that the androgen production was limited to one side. However, bilateral ovarian sampling revealed bilateral adenoma production of androgens. Bilateral oophorectomy was deemed the next course of action to treat the virilization and androgenization. However, the patient was first referred to fertility services to investigate options for conception before the operation was scheduled but patient did not follow up and was lost to care. **LM**

References

1. Tutzer M, Winnykamien I, Davila Guardia J, Castelo-Branco C. Hyperandrogenism in post-menopausal women: a diagnosis challenge. *Gynecol Endocrinol*. 2014;30(1):23–25.
2. Collett-Solberg P. Congenital adrenal hyperplasia: from genetics and biochemistry to clinical practice, Part I. *Clin Pediatr.* 2001;40(1):1–16.
3. Bidet M, Bellanné-Chantelot C, Galand-Portier M-B, et al. Fertility in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab.* 2010;95(3):1182–1190.
4. Burger HG. Androgen production in women. *Fertil Steril.* 2002;77(Suppl 4):S3–S5.
5. Bailey AP, Schutt AK, Carey RM, Angle JF, Modesitt SC. Hyperandrogenism of ovarian etiology: utilizing differential venous sampling for diagnosis. *Obstet Gynecol.* 2012;120(2 Pt 2):476–479.

DOI: 10.1093/labmed/lmaa032