INTRODUCTION

Coagulation tests, mainly including prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), thrombin time (TT), and fibrinogen (FIB), are widely used in the clinic for anticoagulant monitoring, evaluation of hemorrhage, and routine preoperative screening.\(^1\)\(^2\) It is important to establish reference intervals to evaluate coagulation test results. Recently, associations between ABO blood type and coagulation test values have been shown. It was reported that there is a higher aPTT value in healthy volunteers with type O blood than in those with the non-O blood types,\(^3\)\(^-\)\(^5\) while PT does not differ among ABO blood groups.\(^5\)

This study aims to investigate the reference ranges of coagulation tests for different ABO blood groups in the Han population in South China.
using reference intervals. Reference intervals for coagulation tests did not consider the influence of ABO blood type on test results. Therefore, it is essential to establish ABO blood type-specific reference intervals for coagulation tests.

Although there are 56 ethnic groups in China, the Han ethnic group accounted for 91.11% of the total population in 2020 during the seventh national census. In Chaoshan District, 99% of the population was of the Han ethnic group. This study aimed to investigate the reference ranges of coagulation tests for ABO blood groups among the Han population in Chaoshan District of Guangdong Province in South China. Furthermore, the influences of age and sex on the results of coagulation tests were also investigated.

2 | METHODS

A retrospective descriptive study was conducted in the First Affiliated Hospital of Shantou University Medical College. Between January 2015 and September 2019, coagulation testing was continuously reviewed, and results were collected from inpatients aged between 20 and 79 years who had not been diagnosed with diseases, including acute infections, severe hepatic or renal dysfunction, and coagulation abnormalities. The individuals were divided into groups according to their ABO blood type, age, and sex. A total of 9600 individuals were included. Coagulation tests included PT, INR, aPTT, TT, and FIB. The study was approved by the ethics committee of the First Affiliated Hospital of Shantou University Medical College (No. B-2021-203).

| **TABLE 1** Coagulation assays of healthy adults with ABO blood types |
|----------------|---------|---------|---------|---------|---------|
| **ABO blood types** | **A** | **B** | **AB** | **O** | **p Value** |
| **Total number** | 2400 | 2400 | 2400 | 2400 | 0.980 |
| **Age (years)** | 49.438 ± 17.223 | 49.260 ± 17.133 | 49.327 ± 16.989 | 49.433 ± 17.034 |
| **PT (s)** | 11.143 ± 0.607 | 11.160 ± 0.606 | 11.125 ± 0.599 | 11.208 ± 0.634 | 0.000 |
| **95% reference range** | 10.000–12.400 | 10.000–12.500 | 10.000–12.400 | 10.100–12.500 |
| **Male** | 11.195 ± 0.631 | 11.163 ± 0.607 | 11.134 ± 0.592 | 11.205 ± 0.631 | 0.020 |
| **Female** | 11.091 ± 0.578 | 11.157 ± 0.604 | 11.116 ± 0.606 | 11.211 ± 0.636 |
| **INR** | 0.969 ± 0.053 | 0.971 ± 0.054 | 0.967 ± 0.053 | 0.975 ± 0.056 | 0.000 |
| **95% reference range** | 0.870–1.089 | 0.870–1.100 | 0.870–1.080 | 0.880–1.100 |
| **Male** | 0.974 ± 0.055 | 0.971 ± 0.054 | 0.968 ± 0.052 | 0.975 ± 0.055 | 0.011 |
| **Female** | 0.964 ± 0.051 | 0.970 ± 0.054 | 0.967 ± 0.054 | 0.975 ± 0.056 |
| **aPTT (s)** | 28.289 ± 2.569 | 28.251 ± 2.529 | 28.116 ± 2.547 | 29.428 ± 2.553 | 0.000 |
| **95% reference range** | 24.400–33.700 | 24.400–33.500 | 24.300–33.700 | 25.300–34.300 |
| **Male** | 28.272 ± 2.558 | 28.211 ± 2.532 | 28.242 ± 2.601 | 29.468 ± 2.508 | 0.000 |
| **Female** | 28.306 ± 2.581 | 28.291 ± 2.528 | 27.991 ± 2.488 | 29.388 ± 2.598 | 0.000 |
| **TT (s)** | 17.796 ± 1.437 | 17.718 ± 1.427 | 17.779 ± 1.512 | 17.777 ± 1.453 | 0.268 |
| **95% reference range** | 15.102–20.700 | 14.900–20.600 | 14.900–20.800 | 14.900–20.700 |
| **Male** | 17.775 ± 1.438 | 17.643 ± 1.431 | 17.739 ± 1.508 | 17.750 ± 1.482 | 0.132 |
| **Female** | 17.816 ± 1.435 | 17.792 ± 1.420 | 17.819 ± 1.515 | 17.805 ± 1.424 | 0.969 |
| **FIB (g/L)** | 2.973 ± 0.547 | 2.973 ± 0.561 | 2.971 ± 0.551 | 2.980 ± 0.558 | 0.947 |
| **95% reference range** | 2.080–3.960 | 2.060–3.989 | 2.040–3.960 | 2.070–3.960 |
| **Male** | 3.003 ± 0.556 | 2.996 ± 0.560 | 3.003 ± 0.561 | 2.975 ± 0.564 | 0.590 |
| **Female** | 2.943 ± 0.536 | 2.951 ± 0.561 | 2.940 ± 0.539 | 2.985 ± 0.552 | 0.158 |

Note: Values are shown as the mean ± standard deviation.

Abbreviations: aPTT, activated partial thromboplastin time; FIB, fibrinogen; INR, international normalized ratio; PT, prothrombin time; TT, thrombin time.

O blood group vs. A, B, and AB blood groups, \( p < 0.05 \).

AB blood group vs. A and B blood groups, \( p < 0.05 \).
| Gender | Male | Female |
|--------|------|--------|
|        | A    | B      | AB    | O     | p Value |
|        |      |        |       |       |         |
| **Total number** | 1200 | 1200 | 1200 | 1200 |         |
| **PT (s)** |      |        |       |       |         |
| 20–29  | 11.229 ± 0.625 | 11.186 ± 0.588 | 11.142 ± 0.595 | 11.212 ± 0.628 | 0.509 |
| 30–39  | 11.155 ± 0.613 | 11.074 ± 0.589 | 11.116 ± 0.584 | 11.236 ± 0.616 | 0.049 |
| 40–49  | 11.181 ± 0.676 | 11.133 ± 0.576 | 11.189 ± 0.617 | 11.186 ± 0.655 | 0.795 |
| 50–59  | 11.068 ± 0.624 | 11.092 ± 0.627 | 11.139 ± 0.601 | 11.134 ± 0.593 | 0.599 |
| 60–69  | 11.221 ± 0.624 | 11.230 ± 0.621 | 11.137 ± 0.599 | 11.183 ± 0.631 | 0.422 |
| 70–79  | 11.315 ± 0.604 | 11.262 ± 0.624 | 11.082 ± 0.554 | 11.280 ± 0.661 | 0.001 |
| **INR** |      |        |       |       |         |
| 20–29  | 0.976 ± 0.054 | 0.972 ± 0.052 | 0.969 ± 0.052 | 0.975 ± 0.055 | 0.542 |
| 30–39  | 0.970 ± 0.054 | 0.963 ± 0.052 | 0.966 ± 0.051 | 0.978 ± 0.055 | 0.038 |
| 40–49  | 0.973 ± 0.060 | 0.968 ± 0.051 | 0.973 ± 0.054 | 0.973 ± 0.057 | 0.718 |
| 50–59  | 0.963 ± 0.055 | 0.964 ± 0.055 | 0.969 ± 0.052 | 0.967 ± 0.053 | 0.741 |
| 60–69  | 0.975 ± 0.055 | 0.978 ± 0.054 | 0.968 ± 0.053 | 0.973 ± 0.055 | 0.355 |
| 70–79  | 0.984 ± 0.053 | 0.979 ± 0.057 | 0.964 ± 0.049 | 0.982 ± 0.057 | 0.001 |
| **aPTT (s)** |      |        |       |       |         |
| 20–29  | 28.932 ± 2.620 | 28.507 ± 2.589 | 28.518 ± 2.664 | 30.110 ± 2.603 | 0.000 |
| 30–39  | 27.959 ± 2.602 | 28.089 ± 2.533 | 28.017 ± 2.581 | 29.357 ± 2.441 | 0.000 |
| 40–49  | 28.192 ± 2.445 | 27.881 ± 2.492 | 28.220 ± 2.762 | 29.368 ± 2.354 | 0.000 |
| 50–59  | 27.816 ± 2.347 | 28.021 ± 2.364 | 28.298 ± 2.641 | 29.126 ± 2.674 | 0.000 |
| 60–69  | 28.036 ± 2.584 | 28.291 ± 2.583 | 28.033 ± 2.433 | 29.189 ± 2.418 | 0.000 |
| 70–79  | 28.695 ± 2.574 | 28.476 ± 2.589 | 28.365 ± 2.507 | 29.662 ± 2.443 | 0.000 |
| **TT (s)** |      |        |       |       |         |
| 20–29  | 17.996 ± 1.547 | 17.798 ± 1.372 | 17.813 ± 1.510 | 17.897 ± 1.559 | 0.527 |
| 30–39  | 17.560 ± 1.361 | 15.584 ± 1.431 | 17.770 ± 1.546 | 17.704 ± 1.563 | 0.439 |
| 40–49  | 17.785 ± 1.492 | 17.555 ± 1.431 | 17.765 ± 1.455 | 17.847 ± 1.438 | 0.208 |
| 50–59  | 17.810 ± 1.359 | 17.759 ± 1.490 | 17.804 ± 1.483 | 17.770 ± 1.419 | 0.980 |
| 60–69  | 17.815 ± 1.383 | 17.637 ± 1.388 | 17.696 ± 1.481 | 17.745 ± 1.478 | 0.646 |
| 70–79  | 17.687 ± 1.459 | 17.528 ± 1.467 | 17.590 ± 1.578 | 17.540 ± 1.418 | 0.700 |

(Continues)
Data are expressed as the mean ± SD. Differences between group means were assessed by an unpaired Student’s t test for single comparisons or by ANOVA for multiple comparisons using SPSS 25.0. A p value < 0.05 was considered to indicate statistical significance.

3 | RESULTS

Among 9600 individuals with available ABO blood types, there were 2,400 people in each ABO blood group. As shown in Table 1, differences in age, TT, and FIB between ABO blood groups were not significant. Individuals with type O blood had prolonged PT and INR compared with individuals with non-O blood types. There were no differences in PT and INR between the A, B, and AB blood groups. The differences in PT and INR between females and males were significant in the type A blood group but not in other blood groups. Although there were higher values of PT and INR in individuals with type O blood than in those with non-O blood types, the differences were approximately 0.08 s and 0.008, respectively, which may have no clinical significance.

It has been demonstrated that individuals with type O blood have a higher aPTT than those with non-O blood types.3–5 Furthermore, aPTT varied with race. In the present study, individuals with type O blood also had significantly higher aPTT values than individuals with types A, B, and AB blood (29.428 ± 2.553 s vs. 28.289 ± 2.569 s, 28.251 ± 2.567 s, and 28.116 ± 2.547 s, respectively, p < 0.05). There was no difference in aPTT among the A, B, and AB blood groups. The 95% reference ranges of aPTT in the blood groups of A, B, AB, and O were 24.400 ~ 33.700 s, 24.400 ~ 33.500 s, 24.300 ~ 33.700 s, and 25.300 ~ 34.300 s, respectively. aPTT was affected by sex in blood group AB but not in the other ABO blood groups, with higher values in males than in females (as shown in Table 1).

The concentrations of most coagulation proteins vary significantly with age.9 It was reported that aPTT was prolonged in infants9 and children10 compared with adults.

To investigate the effect of age on coagulation tests, coagulation assays were analyzed based on ages from 20 to 79 (as shown in Table 2). There were significant age-related differences in aPTT in all ABO blood groups in females and in the A and O blood groups in males. Age-, sex-, and ABO-specific reference intervals for aPTT, PT, INR, TT, and FIB in the A, B, AB, and O blood groups are shown in Figures 1–4.

4 | DISCUSSION

Coagulation tests are widely used in the clinic to detect hemostatic abnormalities and in preoperative evaluations. PT, aPTT, TT, INR, and FIB are commonly performed coagulation tests. Reference intervals are used to decide whether coagulation tests are normal or abnormal. Many factors, such as biological factors and methodological factors, affect reference intervals11; therefore, it is necessary to
standardize these factors. However, it is impossible to eliminate the influence of sex, age, race, and similar factors on reference intervals, and dividing reference intervals into appropriate groups is important. Coagulation tests are also affected by sex, age, race, and physiological differences such as gestation. Reference intervals of coagulation tests have been reported for pediatrics, adults, and gestation among different ethnic groups.

Recently, associations between ABO blood groups and coagulation tests have been shown. It was reported that there were significantly prolonged values of aPTT in adults with type O blood than in those with non-O blood types, while the difference in PT among ABO blood groups was not significant. However, the sample sizes in these studies were small, and reference intervals for different ABO blood groups were not known. Establishing reference intervals with large sample sizes is needed.

In the present study, we evaluated coagulation tests in 9600 Chinese individuals. We found that the individuals with blood type O had prolonged aPTT compared with those with non-O blood types.
aPTT in individuals aged between 20 and 79 with type O blood was higher than in those with non-O blood types, in both males and females. aPTT was significantly higher in males than in females in the AB blood group. The study showed that ABO blood type-specific reference values of aPTT are essential for the accurate interpretation of coagulation test results with individuals of different ABO blood groups.

There were higher PT and INR in the individuals with blood type O than in those with non-O blood types in the present study, which is discordant with the previous study by Choi Q et al. in 2015, showing that PT was not affected by ABO blood group. The mean PT and INR differed by approximately 0.08 s and 0.008, respectively, in those with type O blood versus those with type non-O blood. It seems that significant differences in PT and INR among ABO blood groups were associated with the large sample size in the present study, which allows for the detection of minor variations. This difference may have no clinical significance.

The mechanisms of higher aPTT in individuals with blood type O are not well known. ABO blood type is associated with von
Willebrand factor (vWF) and factor (F) VIII, with lower levels of vWF and FVIII in people with blood type O than in those with non-O blood types.\textsuperscript{18,19} It was reported that increased FVIII can shorten aPTT,\textsuperscript{20} which suggested that variations in aPTT can be partly explained by the ABO type.\textsuperscript{5}

There were some limitations in the present study. First, it was a retrospective study, and coagulation tests were collected from inpatients, which may differ from a healthy population. We cannot exclude unknown factors affecting coagulation test results. Second, the individuals in the present study were aged from 20 to 79 years old. It was reported that the median time of aPTT and TT was significantly prolonged in all pediatric age groups compared with the adult group in a previous study\textsuperscript{10}; therefore, reference values of the individuals aged below 20 years remained to be determined. Finally, due to race-based differences in aPTT,\textsuperscript{21} the reference range of coagulation assays in a healthy population in the Chaoshan region may be different from that in other populations in China.
CONCLUSION

In summary, the present study established ABO blood type-specific reference intervals for coagulation tests and provided age- and sex-related reference intervals. The reference intervals in the present study showed significant variation in aPTT with ABO blood groups, suggesting that ABO blood groups should be evaluated using reference ranges of coagulation tests.

ACKNOWLEDGEMENTS

This work was supported by the Medical and Health Science and Technology Planning Project of Shantou (Fourth Project in 2021) and the Medical Scientific Research Foundation of Guangdong Province (No A2018125).

CONFLICT OF INTEREST

There are no conflicts of interest.
AUTHORS CONTRIBUTIONS
ZLC drafted the manuscript. XQD and JC performed the experiments. XRT analyzed the data. SYC and MY conceived the study and edited the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author.

ORCID
Min Yu https://orcid.org/0000-0003-1135-5088

REFERENCES
1. Kamal AH, Tefferi A, Pruthi RK. How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. Mayo Clin Proc. 2007;82(7):864-873.
2. Van Geffen M, van Heerde WL. Global haemostasis assays, from bench to bedside. Thromb Res. 2012;129(6):681-687.
3. Fourel V, Gabastou JM, Desroys du Roure F, Ehrhardt N, Robert A. Influence of age, sex and ABO blood group on activated partial thromboplastin time. Haemostasis. 1993;23(6):321-326.
4. Fatima M, Rajkumari CN. Influence of ABO blood groups on activated partial thromboplastin time. Inter J Physiol. 2017;5(1):60-65.
5. Choi Q, Kim JE, Kim SY, Han KS, Kim HK. Influence of ABO type on global coagulation assay results: effect of coagulation factor VIII. Clin Chem Lab Med. 2015;53(9):1425-1432.
6. Castellone DD. Establishing reference intervals in the coagulation laboratory. Int J Lab Hematol. 2017;39:121-127.
7. Ozarda Y, Sikaris K, Streichert T, Macri J. IFCC Committee on Reference intervals and Decision Limits (C-RIDL). Distinguishing reference intervals and clinical decision limits - A review by the IFCC committee on reference intervals and decision limits. Crit Rev Clin Lab Sci. 2018;55(6):420-431.
8. http://www.stats.gov.cn/tjjs/zdtjhz/dqcrkpc/dqcrkpc/ggl/202105/t20210519_1817695.html
9. Andrew M, Paes B, Milner R, et al. Development of the human coagulation system in the full-term infant. Blood. 1987;70(1):165-172.
10. Liu J, Dai Y, Yuan E, et al. Paediatric reference intervals for common coagulation assays in Chinese children as performed on the STA-R coagulation analyzer. Int J Lab Hematol. 2019;41(5):697-701.
11. Solberg HE, PetitClerc C. Approved recommendation (1988) on the theory of reference values. Part 3. Preparation of individuals and collection of specimens for the production of reference values. Clin Chim Acta. 1988;177(3):53-511.
12. Liu J, Yuan E, Lee L. Gestational age-specific reference intervals for routine haemostatic assays during normal pregnancy. Clin Chim Acta. 2012;413(1-2):258-261.
13. Zhang GM, Zhang W, Zhang GM. Age-specific reference intervals for PT, aPTT, fibrinogen and thrombin time for parturient women. Thromb Haemost. 2019;119(6):894-898.
14. Zhang H, Li J, Chen H, Wu X. Establishing reference intervals of coagulation indices based on the ACL Top 700 system for children in Southwestern Fujian, China. Clin Biochem. 2020;75:78-82.
15. Sung JY, Seo JD, Ko DH, et al. Establishment of pediatric reference intervals for routine laboratory tests in Korean population: a retrospective multicenter analysis. Ann Lab Med. 2021;41(2):155-170.
16. Arslan FD, Serdar M, Merve Ari E, et al. Determination of age-dependent reference ranges for coagulation tests performed using destiny plus. Iran J Pediatr. 2016;26(3):e6177.
17. Zierk J, Ganslandt T, Rauh M, Metzler M, Strasser E. Data mining of reference intervals for coagulation screening tests in adult patients. Clin Chim Acta. 2019;499:108-114.
18. Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L, Favaloro JW. Reassessment of ABO blood group, sex, and age on laboratory parameters used to diagnose von Willebrand disorder: potential influence on the diagnosis vs the potential association with risk of thrombosis. Am J Clin Pathol. 2005;124(6):910-917.
19. Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L. Cross-laboratory audit of normal reference ranges and assessment of ABO blood group, gender and age on detected levels of plasma coagulation factors. Blood Coagul Fibrinolysis. 2005;16(8):597-605.
20. Mitsuguro M, Okamoto A, Shironouchi Y, et al. Effects of factor VIII levels on the aPTT and anti-Xa activity under a therapeutic dose of heparin. Int J Hematol. 2015;101(2):119-125.
21. Ho P, Ng C, Rigano J, et al. Significant age, race and gender differences in global coagulation assays parameters in the normal population. Thromb Res. 2017;154:80-83.

How to cite this article: Chen Z, Dai X, Cao J, Tan X, Chen S, Yu M. Reference intervals for coagulation tests in adults with different ABO blood types. J Clin Lab Anal. 2022;36:e24269. doi:10.1002/jcla.24269