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Peripheral blood CD177$^+$ cells as an early diagnostic marker for biliary atresia: A prospective multicentre study in pediatric patients with cholestasis

To the Editor:

The differential diagnosis of early-stage biliary atresia (BA) from other causes of infantile cholestasis is difficult with laboratory examinations, but is important for improving native liver survival and reducing postoperative complications.[1] Gamma-glutamyltransferase (GGT) is commonly used to distinguish BA from non-BA with a cut-off value of 300 U/L, but it has a relatively low accuracy of 76% to 88%.[2] Our recent study showed that a large number of CD177$^+$ cells infiltrated the BA liver and damaged the bile ducts at an early stage,[3] prompting us to investigate the potential of peripheral blood CD177$^+$ cells as an early diagnostic indicator of BA.

Patients from our own hospital were enrolled into a diagnostic cohort and a prospective validation cohort in two time periods. In addition, the patients collected from the other 4 hospitals were used as another prospective validation cohort. In each cohort, patients with cholestasis were separated into BA groups and non-BA groups and compared (Fig. S1 and Table S1). The diagnostic efficacy of CD177$^+$ cells for BA was examined using the ratio of these cells to CD45$^+$ cells in the peripheral blood in the diagnosis cohort. The results showed that the percentage of CD177$^+$ cells in the group of patients with BA was significantly higher, with a median [IQR] level of 25.90 [19.75-31.10] compared to the non-BA group (8.56 [6.01-12.65]) and normal control group (7.15 [4.12-11.13]), respectively, p <0.0001. Compared to the non-BA group, the receiver-operating characteristic (ROC) curve analysis indicated a good diagnostic ability of CD177$^+$ cells, with an area under the curve (AUC) of 0.9601 [95% CI 0.9218-0.9983]. The sensitivity was 0.8297, the specificity was 1.000, the accuracy was 0.9259, the positive predictive value was 1.000 and the negative predictive value was 0.8695. Meanwhile, the ability of the CD177$^+$ cell proportion to discriminate between BA and non-BA was validated in two prospective validation cohorts with AUCs of 0.9482 [95% CI 0.9004-0.9959] and 0.9211 [95% CI 0.8529-0.9892], respectively. In the two prospective validation cohorts, the sensitivities of the proportion of CD177$^+$ cells were 0.9844 and 0.9032; the specificities were 0.8776 and 0.8684; and the accuracies were 0.9345 and 0.8841, suggesting that the CD177$^+$ cell ratio has good diagnostic performance for the differential diagnosis of BA (Fig. 1A and Table S2).

To further explore the potential of CD177$^+$ cells as an early diagnostic indicator of BA, we performed a subanalysis of data collected within 45 days from across the three cohorts, including 21 infants in the BA group, 27 infants in the non-BA group, and 11 in normal controls (Table S3). The results showed that the median proportion of CD177$^+$ cells in the peripheral blood of the patients in the non-BA subgroup (aged less than 45 days) was 11.30 [6.79-14.40], while it was significantly increased to 23.90 [19.75-32.10] (p <0.0001) in the BA group. The ROC analysis performed in this subgroup showed an AUC of 0.9189 (95% CI 0.8266-1.0000; p <0.0001), a sensitivity of 0.9048, a specificity of 0.9259, an accuracy of 0.9167, a positive predictive value of 0.9047, and a negative predictive value of 0.9310 (Fig. 1B), suggesting that CD177$^+$ cells have the potential to differentiate between early-stage BA and non-BA.

For analysis of the diagnostic value of the combination of peripheral blood CD177$^+$ cells and serum GGT for BA, we grouped the three cohorts (a total of 335 individuals) and compared the diagnostic efficacy of BA between the GGT and CD177$^+$ cell proportions by ROC curve analysis. The results showed that the AUC of GGT was 0.8319 (95% CI 0.7845-0.8793; p <0.0001) (Fig. 1C), which was significantly lower than the AUC of CD177$^+$ cell proportion (0.9423; 95% CI 0.9124-0.9722; p <0.0001) (Fig. 1B). Furthermore, the composite model combining the value of CD177$^+$ cells and GGT produced an AUC of 0.9558 (95% CI 0.9319-0.9797; p <0.0001), with a sensitivity of 0.8603 and specificity of 0.8898. In the subgroup of ≤45-day analysis, we also found that the AUC of GGT (0.6825; 95% CI 0.5305-0.8345) was significantly lower than that of CD177$^+$ cells alone. Similarly, the combination of CD177$^+$ cells and GGT showed good diagnostic performance for the subgroup (AUC 0.9121; 0.8186-1.000; p <0.0001) (Fig. 1C).

Collectively, these data suggested that the diagnostic accuracy of CD177$^+$ cells was significantly greater than that of GGT. Compared with CD177$^+$ cells alone, adding the analysis of GGT to the analysis of CD177$^+$ cells does not substantially increase the diagnostic accuracy for BA. Recently, increased expression of matrix metalloproteinase 7 (MMP-7) was suggested to be a potential biomarker of BA, and the combination of MMP-7 and GGT has a relatively stable diagnostic ability with an AUC of 0.94.[4] However, whether it has diagnostic value for early BA is unknown. Additional investigation of the early age subgroup analysis of MMP-7 and in combination with CD177$^+$ cells and GGT tests is worth performing in the future.

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Conflicts of interest

Authors declare no conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

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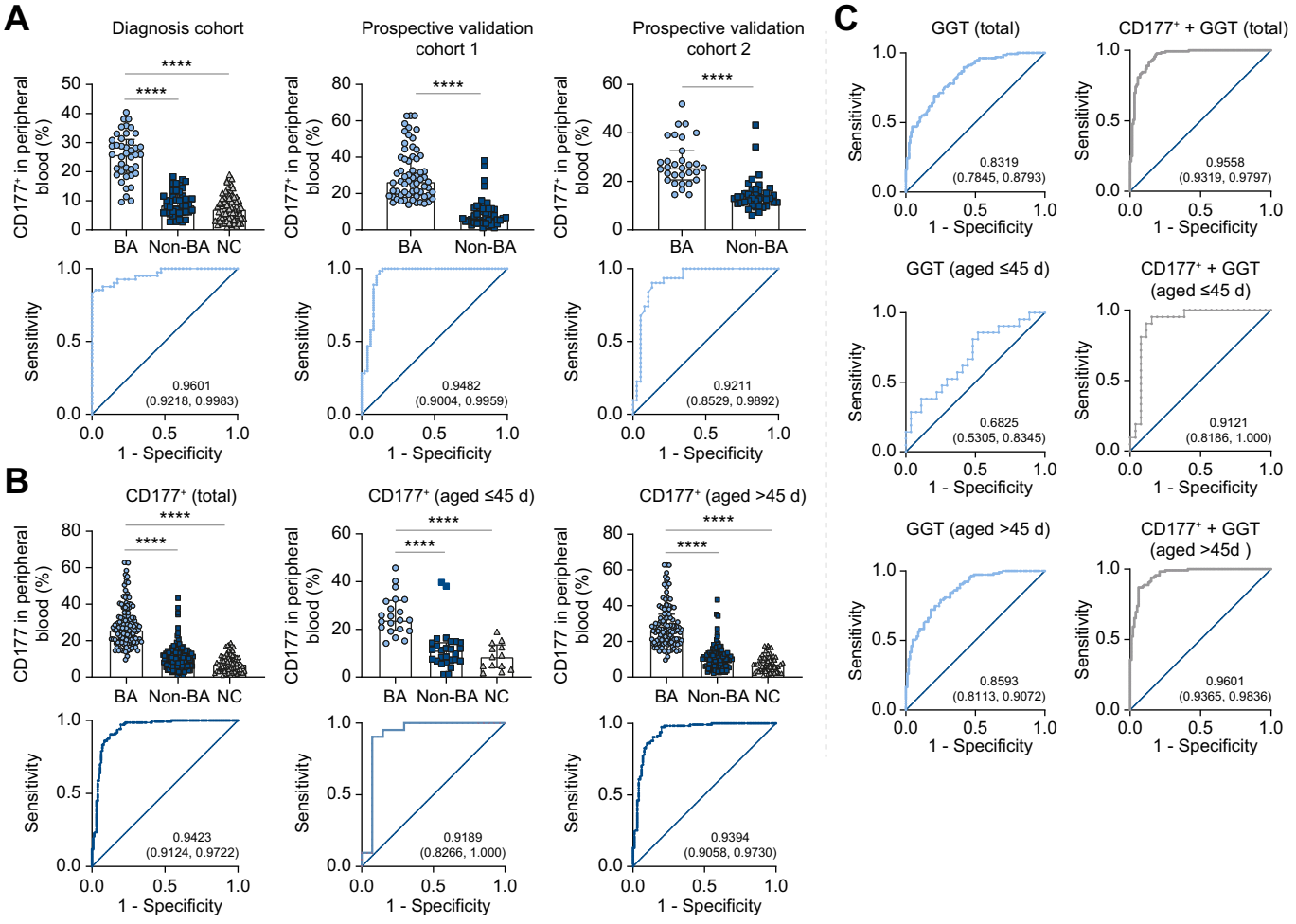

Fig. 1. Diagnostic value of peripheral blood CD177⁺ cells for the differentiation of BA and non-BA patients. (A) Expression levels and ROC curves of CD177⁺ cells in the BA, non-BA and NC groups in the diagnostic cohort, prospective validation cohort 1 and cohort 2. Scatter plots (upper panel), ROC curves and AUCs (lower panel) are shown (B) CD177⁺ cells in the age subgroups compared between patients with BA (n = 136), those without BA (n = 127) and healthy controls (n = 72). Scatter plot (upper panel), ROC curve and AUC (lower panel) of the CD177⁺ cell proportion are shown (C) ROC curve of combined serum GGT and peripheral blood CD177⁺ cell proportions compared with the serum GGT alone in BA diagnosis. ROC curve and AUC of the BA and non-BA groups from 3 cohorts (upper panel), aged ≤45 days subgroups (middle panel) and age >45 days subgroup (lower panel). AUC, area under the curve; BA, bile acid; GGT, gamma-glutamyltransferase; NC, healthy control; ROC, receiver-operating characteristic.

Authors' contributions

R.Z. designed the experiments and recruited patients. J.Y. was responsible for samples collection and flow analysis. J.R. performed statistical analysis. Y.C. and H.X. wrote the letter and supervised the project.

Clinical trial registration

Peripheral blood CD177⁺ cell as early diagnostic markers for biliary atresia: multicenter study (ChiCTR1800015717).

Ethical statement

The study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center, China.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.08.005.

Appendix

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New terms for fatty liver disease other than MAFLD: Time for a reality check

To the Editor:

In 2020 a landmark consensus by international experts put forth a comprehensive proposal for renaming and defining what was previously termed non-alcoholic fatty liver disease (NAFLD). The conceptualised framework changed the disease from one that was diagnosed when all other causes for hepatic steatosis were excluded (hence removing the "non" word) and its overemphasis on alcohol. The new term metabolic (dysfunction) associated fatty liver (MAFLD) and its diagnostic criteria acknowledged the dominant role of metabolic dysregulation in disease pathogenesis. Despite the multiple positive attributes that have been supported by mounting evidence of its superior clinical utility, controversy still exists.[1–5] Importantly, during the course of this debate NAFLD and MAFLD remain the only two alternatives for the disease nomenclature.

Recently, multiple potential alternative terms have been proposed. From our perspective, it is time for a reality check to distinguish the genuine from the hype. First of all, how did MAFLD come about? A group of 31 experts engaged in a 2 round Delphi process to consider alternative names, recognising that NAFLD for various reasons did not serve the field. In the first round, experts were asked to come up with any and all alternative terms (*i.e.* an open question). In the second round, the panel voted on their top 6 choices from the names suggested in round one. In round two, MAFLD was supported by ~70% of participants.[5] This data was published as a "proposed" nomenclature after which extensive clinical research ensued across the world, validating the name and its diagnostic criteria.[4] Hence, one could legitimately ask what can we expect from repeating the same process again apart from feeding a sense of confusion and division. Notably, none of the other terms proposed have been accompanied by a set of diagnostic criteria for adults and children that tells us what the disease is. Rather, what all the other terms have in common is the "non"-definition of NAFLD clothed in a term that does not have the word "non" and perhaps "alcohol".

Second, it should be noted that disagreement around MAFLD may be less than initially assumed and has focused on a small but vocal group interested in maintaining the status quo. MAFLD has received substantial endorsement from multiple pan-national and national societies, and stakeholders.[6] At the apex, >1,000 stakeholders including hepatologists, endocrinologists, paediatricians, primary-care providers, pathologists, patient advocates, nurses, nutritionists, and pharmaceutical experts from >134 countries endorsed the term.[7] Does this not say something?

Third, querying PubMed has shown that >10% of publications since coinage of MAFLD have opted to use that term (this does not include articles that have mentioned both terms), which is double that from the year before. If the same trends hold, within 2 years approximately 40-50% of publications will be using MAFLD not NAFLD. By analogy, the terms NAFLD/NASH took over 15 years from when first coined to reach to the same number of publications that MAFLD has reached in 2 years. This has been in the context of substantial barriers at the publication interface.

The number of citations gives another proxy of academic acceptance. The two original publications on MAFLD[4,5] are cited over 12.4 fold more than the two opposing editorials[8,9] (2,373 *vs.* 191 cites, respectively as per 18 July 2022). In addition, we

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