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Suitability of EDTA-anticoagulated Blood for Natural Killer Cell Activity Testing Using Flow Cytometry

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Dear Editor,

Natural killer (NK) cell activity is measured to assess innate or anti-tumor immunity and the effect of donor lymphocyte infusion and to diagnose hemophagocytic lymphohistiocytosis [1-6]. Flow cytometry is widely used to measure NK cell activity owing to its simplicity, reproducibility, and good correlation with the chromium release assay, which is the reference method [3, 4]. Heparin is preferred over EDTA as an anticoagulant because the latter reduces NK cell cytotoxic activity owing to its calcium chelating property [5]. Each test run requires samples from healthy individuals to serve as internal QCs and for calculating NK cell activity [7]; however, obtaining fresh heparin-anticoagulated control samples is not feasible. The use of leftover heparin-anticoagulated blood is not practical because it is limited to only a few tests, such as cytogenetic assays for individuals suspected of having hereditary diseases. In contrast, residual EDTA-anticoagulated samples from healthy individuals are essentially ever-present in clinical laboratories as they are used for various tests frequently requested as part of regular health checkups that are actively performed in Korea. Son, *et al.* [8] showed that immediate washing of peripheral blood mononuclear cells (PBMCs) extracted from EDTA-anticoagulated samples minimized the effect of EDTA, rendering the activity of NK cells comparable to that of their counterparts in heparin-anticoagulated samples. Therefore, this study newly revealed that leftover EDTA-anticoagulated samples can replace heparin-anticoagulated counterparts for purposes of testing NK cell activity using flow cytometry. The study was approved by the ethics committee of Asan Medical Center, Seoul, Korea (approval No. 2022-1048), and the requirement for written informed consent was waived by the committee.

The study was conducted at Asan Medical Center from 7, 2021 to 6, 2022. Among the patients from whom EDTA-anticoagulated samples were collected for other tests, heparin-anticoagulated samples were collected from six of those who were referred for the NK cell activity test. The study consisted of two steps. First, NK cell activity values derived from heparin- and EDTA-anticoagulated blood samples obtained from the same six patients were compared. Second, NK cell activity values were determined in 30 ABO-matched, EDTA-anticoagulated pooled leftover samples from 92 healthy individuals who visited Asan Medical Center for a regular health checkup. The maximum storage time of leftover samples was 4 hours, and the required minimum volume was 10 µL. The results of the EDTA-anticoagulated pooled samples were compared against the reference interval established using heparin-anticoagulated blood [7]. NK

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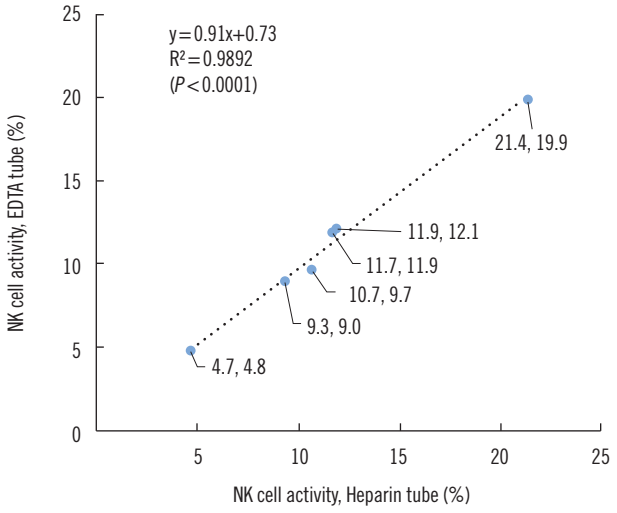

Fig. 1. Comparison of NK cell activity in six paired heparin- and EDTA-anticoagulated samples submitted for flow-cytometric NK cell activity testing.
Abbreviation: NK, natural killer.

cell activity was tested as previously described [7]. K562 human erythroleukemia cells (American Type Culture Collection, Rockville, MD, USA), the target cells, were labeled with fluorescein isothiocyanate (Sigma, St. Louis, MO, USA). To prepare the effector (i.e., NK) cells, PBMCs were separated by centrifugation using Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) from heparin- or EDTA-anticoagulated whole blood and immediately washed with Roswell Park Memorial Institute 1640 medium (Gibco-BRL, Paisley, UK) three times. The cells were co-incubated with the effector cells at an effector-to-target cell ratio of 100:1; dead K562 cells were labeled with propidium iodide (Sigma). Flow-cytometric analysis was performed using a FACSLyric instrument (Becton Dickinson, San Jose, CA, USA) and the FACSuite software (Becton Dickinson). NK cell activity was calculated as previously described [7]. Each sample was incubated twice, and the results were averaged. NK cell activity values derived from heparin- and EDTA-anticoagulated samples were compared directly to calculate the percentage difference and via a correlation equation to calculate R^2.

The NK cell activity values derived from heparin- and EDTA-anticoagulated samples correlated well (Fig. 1). The activity in the six heparin-anticoagulated samples ranged from 4.7% to 21.4% (mean, 11.6%), whereas that in the EDTA-anticoagulated samples ranged from 4.9% to 19.9% (mean, 11.2%). The percentage difference ranged from 1.7% to 9.7% (mean, 4.6%). The majority of EDTA-anticoagulated samples from healthy individuals exhibited NK cell activity that fell within the reference range previously established using heparin-anticoagulated samples [7], supporting our hypothesis that NK cell activity in EDTA-anticoagulated blood from healthy individuals would not significantly differ from that in heparin-anticoagulated blood. The NK cell activity in the 30 pooled samples ranged from 6.7% to 25.8% (mean, 17.2%). For 29 of these samples (96.7%), the values fell within the range of 11.8–31.9%; the sole outlier had a value of 6.7%.

Our findings indicate that leftover EDTA-anticoagulated blood samples can replace heparin-anticoagulated blood samples for NK cell activity testing using flow cytometry. This will eliminate the need to search for healthy donors or draw additional blood from patients, thus facilitating testing in clinical settings. Additional studies with larger numbers of EDTA-anticoagulated samples would help establish age- and sex-specific reference ranges for each laboratory.

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AUTHOR CONTRIBUTIONS

Ryu J collected the information and wrote the manuscript; Choi J performed the NK cell activity test; Kim M designed the study and supervised the manuscript; Cho YU, Hwang SH, and Jang S contributed to the design of the study and reviewed the data. Park CJ designed the NK cell activity test, established the reference intervals with heparinized blood, contributed to the design of this study, and reviewed the data. All authors reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

None declared.

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