Ethylenediamine Tetraacetic Acid-Associated Leukoagglutination

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Three cases of ethylenediamine tetraacetic acid (EDTA)-induced leukoagglutination noted on peripheral blood films are reported. Two cases of EDTA-induced agglutination of benign lymphocytes, and one case of EDTA-induced mature neutrophil satellitosis about immature neutrophils were observed. EDTA-induced agglutination of malignant lymphoid cells has been reported in blood films from patients with malignant lymphoma and chronic lymphocytic leukemia. Our two cases are the first reported instances of EDTA-induced agglutination of benign lymphocytes. EDTA-induced agglutination of neutrophils is a well recognized, but uncommon event. This case was unusual because mature neutrophils were rosetted about a central immature granulocyte and no agglutination of mature neutrophils was noted. (Key words: EDTA; Leukoagglutination; Neutrophils; Lymphocytes; Blood films; EDTA) Am J Clin Pathol 1995; 103:338-340.

Agglutination of platelets, neutrophils, malignant lymphocytes, and platelet satellitosis about neutrophils has been reported in blood exposed to ethylenediamine tetraacetic acid (EDTA). 1-10 This phenomenon occurs in EDTA-anticoagulated blood samples and generally does not occur when the same samples are collected in sodium citrate anticoagulant solutions or in a heparinized tube. The phenomenon also is not observed in a blood film made directly from finger-stick, heel-stick or otherwise non-anticoagulated blood. The incidence of EDTA-induced agglutination of platelets is approximately 1% of routine complete blood count samples. Platelet satellitosis in which the platelets are attached to the periphery of the neutrophils is less common. Agglutination of neutrophils is much less frequent. Clumping of lymphoma cells in the peripheral blood secondary to EDTA has been previously reported. 1 EDTA-induced lymphocyte agglutination has also been reported in chronic lymphocytic leukemia. 2 We observed two cases in which EDTA-induced clumps of normal lymphocytes were present. We also describe a unique case of EDTA-induced neutrophil clumping in which the mature neutrophils are rosetted about immature granulocytes, but do not exhibit agglutination with each other.

Case 1

An 82-year-old white female was admitted to the hospital with a 3-day history of left flank pain, chills, fever, nausea, and vomiting. Urinalysis revealed 2+ bacteria, 3 to 4 red cells and 1 to 2 leukocytes per high power field. Serum creatinine was 1.8 mg/dL (normal 0.6–1.2 mg/dL) and BUN was 27 mg/dL (normal 8–23 mg/dL). The patient was thought to have a urinary tract infection. The initial erythroid and platelet values were normal, but the total leukocyte count was 28,000/μL. A peripheral blood film prepared from EDTA(K3) anticoagulated blood revealed clumps of mature lymphocytes. An additional venous blood sample was collected. One portion was collected into a heparin vacutainer tube and one portion in an EDTA(K3) vacutainer tube. A finger-stick blood smear was prepared directly from finger-stick blood without anticoagulation. A peripheral blood film was prepared from the EDTA-anticoagulated and the heparin anticoagulated tubes and stained with Wright's stain. (An automated count was not performed on the blood collected in heparin.) The blood film prepared from the EDTA tube showed aggregates of 10 to 20 mature lymphocytes (Fig. 1). No agglutination of platelets or neutrophils was present. The blood film prepared from the heparin tube showed slight lymphocyte clumping with aggregates of 6 to 14 cells. The finger-stick blood film showed no agglutination of lymphocytes or neutrophils. The usual platelet clumping observed on finger-stick films was present.

Case 2

A 36-year-old white male with AIDS had malignant B-cell lymphoma and no other clinical information was known. A bone marrow aspirate and biopsy was performed to rule out involvement by malignant lymphoma. On admission, he had a white blood cell count of 10,700/μL with 75% neutrophils, 12% lymphocytes, and 13% monocytes. The bone marrow was essentially normal and did not indicate any involvement by malignant lymphoma. The blood film prepared from EDTA(K3) anticoagulated blood revealed clusters of mature appearing lymphocytes. Each cluster contained 9 to 12 cells (Fig. 2). The lymphocytes appeared to be normal, small-to-medium sized mature cells with clumped chromatin and scanty cytoplasm. Immunophenotypic analysis of peripheral blood by flow cytometry revealed a mixture of T cells and polyclonal B lymphocytes. Blood drawn 3 days later showed no clumping of the lymphocytes or other cell types in EDTA (K3) or sodium citrate anticoagulated samples. An automated cell count was not available. In this case, heparinized blood was not collected.

Case 3

A 41-year-old white male with a history of metastatic adenocarcinoma of the colon to bone and liver was admitted to the hospital be-
cause of leg swelling and decreased urine output. On admission, he had a white blood cell count of 25,000/µL with 88% neutrophils, 2% metamyelocytes, 2% lymphocytes, and 8% monocytes. The BUN was 57 mg/dL (normal 8–23 mg/dL), creatinine 2.0 mg/dL (normal 0.6–1.2 mg/dL) and potassium 7.9 mg/L (normal 3.7–5.1 meq/L). The abnormal renal function tests were thought to be due to severe dehydration. He was aggressively hydrated and the potassium fell to 4.8 at the time of discharge. The peripheral blood film prepared from EDTA(K3) anticoagulated blood exhibited rosetting of mature neutrophils about the periphery of immature neutrophils (Fig. 3). No agglutination of mature neutrophils was observed. Nucleated red cells were present, but no rosetting of the neutrophils about the nucleated red cells or other cells such as lymphocytes or monocytes was noted. The leukoerythroblastic blood picture was assumed to be secondary to the metastatic adenocarcinoma. Unfortunately, no bone marrow specimen was obtained and no repeat blood film was available following rehydration of the patient. Heparinized blood was not available for comparison.

DISCUSSION

EDTA-induced platelet satellitism (Fig. 4) and platelet clumping and granulocytic agglutination (Fig. 5) have been reported.1-11 This clumping is usually not observed when the sample is collected in heparin or sodium citrate anticoagulant or the blood film is prepared directly from a finger stick. Cases 1 and 2 demonstrated EDTA-induced agglutination of benign lymphocytes (Figs. 1 and 2). In case 1, there was also slight agglutination in blood collected in heparin. No agglutination was observed on direct finger-stick preparations in cases 1 and 2. Case 3 demonstrated a unique finding not previously described, which was the rosetting of mature neutrophils about immature neutrophils in an EDTA (K3) anticoagulated blood (Fig. 3).

Juneja and colleagues1 reported a case of EDTA-induced agglutination of lymphoma cells in peripheral blood. Bizarro and colleague2 described EDTA-induced lymphocytic clusters in the peripheral blood of a patient with chronic lymphocytic leukemia. Both of these reports illustrate clumping of abnormal lymphocytes in EDTA-anticoagulated peripheral blood. In our cases, there was clumping of normal lymphocytes that has not been previously described. Case 2 had malignant lymphoma and the possibility that the lymphocytes were lymphoma cells was considered. Immunophenotypic analysis of the peripheral blood by flow cytometry indicated only a mixture of T-cells and polyclonal B lymphocytes. No clonal B lymphocyte population was detected. It was concluded that the lymphocytes were nor-
Platelet agglutination may be recognized on blood smears, a typical pattern on a scatterplot abnormality may be missed and may result in spurious thrombocytopenia. The greatest clinical problem caused by the EDTA-induced cell agglutination is due to platelet clumping and the resulting thrombocytopenia. Although this pseudo-thrombocytopenia is usually detected by examining the instrument display, it may be missed and may result in spurious thrombocytopenia. Platelet agglutination may be recognized by a high Y axis takeoff on the leukocyte histogram or the platelet histogram (Coulter Counter Model S-Plus IV, Hialeah, FL).

Although agglutination of leukocytes collected in EDTA is easily identified on blood smears, a typical pattern on a scatterplot has not been recognized. Heparinized blood from patients with EDTA-associated agglutination should not result in smears with agglutination. However we did observe slight agglutination in case 1. Because we did not have heparinized blood for automated cell counts in the three cases in this report, we cannot comment on the presence of scatterplot abnormalities. We presume that leukocyte agglutination would not result in an abnormal scatterplot because the size of the clumps would be greater than the upper threshold of the automated counter for white blood cell detection. The cause of the EDTA-induced platelet clumping is not known, but appears to be related to platelet antibodies that are functional in the presence of EDTA. Although this artifact is irreversible, it is readily identified on smears, and does not occur in smears from blood collected in heparinized tubes. Similarly, EDTA-induced cell agglutination has no specific mechanism, but is usually observed in cases who are acutely or chronically ill, and usually disappears when the patient recovers. Temperature-dependent EDTA-induced agglutination of neutrophils also has been described. Warming of the sample to 37 °C should cause the agglutination to disappear if it is due to cold agglutinins.

In conclusion, the clumping of benign lymphocytes and re-setting of mature neutrophils around immature neutrophils should be added to the list of EDTA-associated clumping of blood elements, which includes platelet clumping and satellitosis, neutrophil clumping, and clumping of lymphomas and leukemic lymphocytes. When these phenomena are observed on a blood film prepared from EDTA-anticoagulated blood, examination of a peripheral blood film prepared from finger-stick or from sodium citrate or heparin-anticoagulated blood may demonstrate that the disorder is related to EDTA. If the agglutination persists in the sodium citrate or heparin samples, a cold agglutinin should be suspected.

Acknowledgment. The authors thank Rosie Marin for typing the manuscript.

REFERENCES