(p=0.57) and this is in accordance with data concerning TPO levels in fulminant hepatitis.[10] Thus, we speculate that probably other factors are of primary importance in the thrombocytopenia of these patients. In conclusion, liver cirrhosis thrombocytopenia seems to be multifactorial and reduced TPO levels may contribute to this condition.

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Platelet aggregation is stimulated by L-asparginase in children with acute lymphoblastic leukemia and in normal individuals

L-asparginase treatment of childhood acute lymphoblastic leukemia (ALL) can be associated with severe thromboembolic complications. A decrease in anticoagulant proteins C, S and antithrombin III (ATIII) has been attributed to the enzyme. We tested the hypothesis that L-asparginase can also have a platelet agonist effect.

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Intensive use of L-asparginase in ALL is credited with producing a definite improvement in response and cure rates.[1] Due to a decreased biosynthesis of proteins C, S, and anti-thrombin III (ATIII),[2,3] its administration can be complicated by severe thrombotic events, which occur with an incidence of between 2.4% to 11.5%.[4,5] Different mutations in these anticoagulant proteins have been identified in ALL patients,[4] but their prevalences are those expected for the study population and, therefore, do not explain the increased incidence of thrombotic events. Indeed, this increased incidence implies additional disease or treatment-related procoagulant factors.[3,4] We postulated that the platelet phase of the coagulation mechanism could be involved in this complex hypercoagulable state through a platelet agonist effect exerted by L-asparginase. Twenty children (≤ 13 years old) newly diagnosed with ALL were included in the study. Informed consent was obtained. The induction of remission protocol included *E. coli* L-asparginase, 6000 IU/m^2/i.m., twice a week for 6 weeks. The control group consisted of nineteen healthy volunteers, with normal platelet counts and bleeding time.

Blood samples were obtained from the ALL children after administration of the third dose of L-asparginase, or once the platelet count had reached at least 150×10^9/L. The 20 mL samples of whole blood were taken into plastic tubes containing 3.8% sodium citrate, maintaining a 9:1 v/v relationship.

Platelet aggregation studies were carried out employing a previously described optical method.[6] Platelet-rich plasma (PRP) was obtained from whole blood by centrifuging the tube at 135g ×15 min at room temperature. Platelet-poor plasma (PPP), for adjusting the platelet count to 250×10^9/L and for setting the aggregometer reading to 0%, was obtained after centrifugation at 1,500g ×15 min.

Platelet responses were studied within the two hours after drawing the blood, maintaining the assay temperature at 37°C and the agitation speed at 1,000 rpm. The following agonists were employed:[6] 10 μM ADP, collagen 2.5 μg/mL, ristocetin 1.5 μg/mL, and 10 μM epinephrine. Saline dilutions of *E. coli* L-asparginase (Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan) to concentrations of 2,500, 5,000 and 10,000 IU/mL in 0.9% physiologic solution, pH 7.4, were employed as agonists in independent assays. For each assay, 50 μL of agonist were added to a plastic cuvette containing 450 μL of the PRP to be tested. The response was quantified as the maximum percentage of aggregation reached.

No difference in aggregation with standard agonists was found between ALL patients and controls (*not shown*). Optical aggregometry did, however, demonstrate a clear agonist effect of L-asparginase on platelets obtained from both leukemic patients and normal individuals, manifested as a potent primary wave of aggregation. Figures 1 and 2 display typical results obtained when platelets from ALL patients were stimulated with L-asparginase dilutions of 5,000 and 10,000 IU/mL, respectively. In these figures,

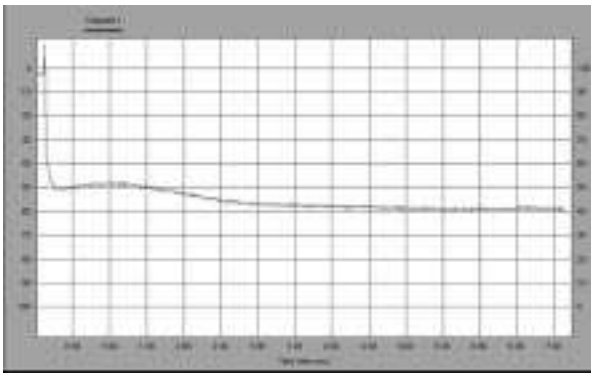

Figure 1. An aggregation response of 60% was obtained after platelets from a child with ALL were stimulated with an *Escherichia coli* L-asparginase dilution of 5,000 IU/mL. The slope was narrow and almost vertical, reflecting the speed of the response.

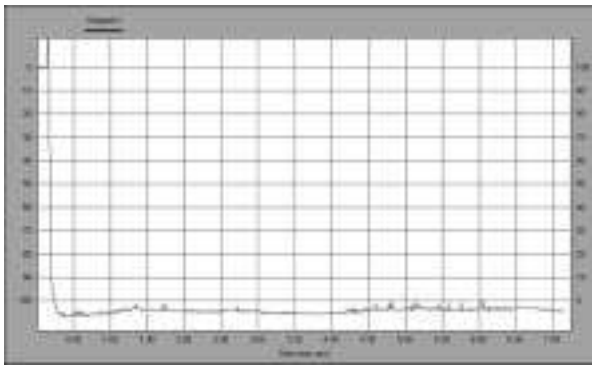

Figure 2. Aggregometric trace obtained after ALL platelets were stimulated with an *Escherichia coli* L-asparginase dilution of 10,000 IU/mL. The vertical slope and the low percentage of disaggregation were characteristic and similar to those observed with the 5,000 IU/mL dilution. The trace is slightly over 100%, reflecting the presence of some residual platelets in the PPP preparation employed to set the initial 100% light transmission reading.

a dose-dependent aggregation response is documented, as the response increased from 60% with the 5,000 IU/mL dilution, to 100% with the 10,000 IU/mL L-asparginase reagent.

E. coli L-asparginase will continue to be the preparation of choice for the initial treatment of childhood ALL. Indeed, it has recently been proven to be significantly superior to *Erwinia* asparaginase in a large multicenter trial, although it caused more than twice the clotting abnormalities.[7] Previous research suggested that L-asparginase can directly attack proteins of the coagulation system *in vitro*;[8] however, to the best of our knowledge, L-asparginase activation of the platelet phase of the coagulation mechanism has not been studied nor has its platelet agonist properties been previously evaluated by optical aggregometry. Our findings demonstrate a direct platelet agonist effect of L-asparginase *in vitro*. If substantiated *in vivo*, the activation of the platelet phase of the coagulation mechanism as an additional consequence of L-asparginase administration could be important in expanding our current understanding of the prothrombotic state observed in children with ALL.

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