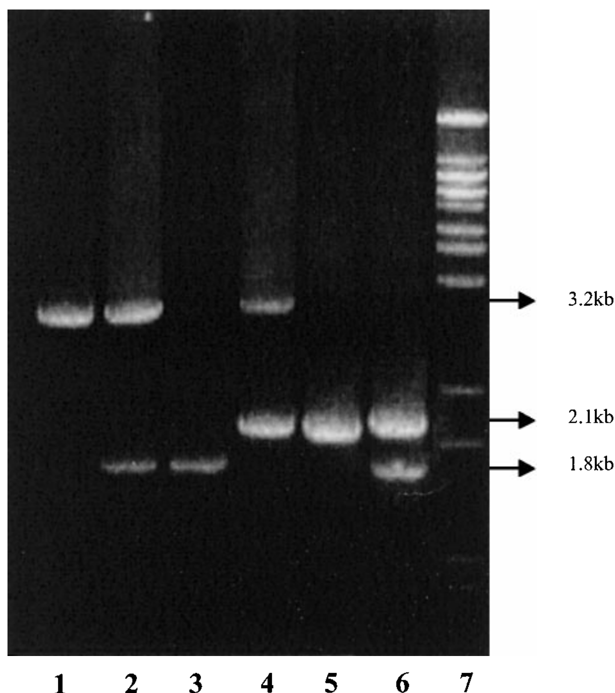

Figure 2. Identification of amplification products by gel electrophoresis. Lane 1: normal ($\alpha\alpha/\alpha\alpha$); 2: heterozygote $-\alpha^{3.7}$ ($-\alpha^{3.7}/\alpha\alpha$); 3: homozygote $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$); 4: heterozygote $-\alpha^{4.2}$ ($-\alpha^{4.2}/\alpha\alpha$); 5: homozygote $-\alpha^{4.2}$ ($-\alpha^{4.2}/-\alpha^{4.2}$); 5: compound heterozygote $-\alpha^{3.7}/-\alpha^{4.2}$; and 7: molecular weight marker.

respectively (Figure 1). Under these experimental conditions, fragments of 5.5 kb and 6.3 kb that would be expected for initiations from A+B and D+E primer pairs, respectively, are not amplified in the PCR reaction.

Standardization of the PCR was carried out with DNA samples of known genotypes: $-\alpha^{3.7}/-\alpha^{3.7}$, $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/-\alpha^{4.2}$, $-\alpha^{4.2}/\alpha\alpha$, and $-\alpha^{4.2}/-\alpha^{3.7}$ (Figure 2).

We conclude that this single-tube multiplex PCR assay for the common α^{+} thalassemia determinants is a simple, rapid, and robust method applicable to large population screening for epidemiological purposes. It will need to be used with caution when applied to diagnosis of individuals with compound heterozygous states for large deletions such as $--^{SEA}/-\alpha^{3.7}$ or $--^{SEA}/-\alpha^{4.2}$, which will be identified as homozygous for the small deletion. Such cases, however, are easily recognized on clinical and hematological features.

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To the editor:

Protein C inhibitor in platelets?

In a recent article, Prendes et al[1] described the presence and localization of protein C inhibitor (PCI) in blood platelets. The concentration of PCI antigen that was determined in platelet lysates was 160 ng/2 $\times$ 10^9 platelets. Although this figure was determined with an extremely accurate ELISA, it was 28 times the previously reported concentration of PCI in platelets.[2] Also, in contrast to what the authors suggest, blood contains approximately 2 $\times$ 10^8 platelets/mL, which implies that the PCI concentration that was determined in the platelet lysate could be due to a contamination of the sample with 0.3% of plasma. Without a proper check for contamination of the platelet lysate with plasma proteins, it is nearly impossible to accurately determine the exact platelet concentration of PCI. Nevertheless, the calculations above already indicate that platelets can hardly influence the concentration of PCI in plasma (5.8 µg/mL).

A second point of concern is the determination of "PCI activity antigen."[1] The authors postulate that platelets contain 14% active PCI antigen relative to plasma. Assuming 100% reactivity of PCI in plasma, the active PCI antigen concentration in 1 mL of blood (equivalent to 2 $\times$ 10^8 platelets) is 810 ng/mL, more than 50 times the PCI antigen concentration in platelets (16 ng/2 $\times$ 10^8 platelets). This could be explained if the "PCI activity antigen" assay was not specific for PCI. If true, this conclusion would undermine the validity of other conclusions in the paper, because the same antibody was also used for immunofluorescence and immunoelectronmicroscopy data.

Finally, the use of PCR to determine mRNA of a protein in platelets should be used cautiously if no control with leukocytes is included. Platelet preparations are nearly always contaminated with leukocytes, and because these contain at least 1000-fold more RNA than platelets, signals are very likely to be from leukocytes instead of platelets.

The physiological role of protein C inhibitor is not yet known. Characterization of knockout mice will be invaluable in determining the relevance of PCI for hemostasis and other systems. However, the role of platelet PCI in hemostasis, if any, is likely to be marginal because of the extremely low concentration in platelets.

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Response:

Protein C inhibitor does exist in platelets

In our study,[1] which was criticized by Dr Meijers, we wanted to determine whether or not platelets contain protein C inhibitor (PCI). It was not the aim to analyze to what extent platelet PCI might influence the overall PCI activity in blood. Using different approaches we can show that PCI is present in platelets. We can furthermore show that PCI contained in platelets originates from synthesis in megakaryocytes as well as from uptake by platelets. Therefore the comment that ''it is nearly impossible to accurately determine the exact platelet concentration of PCI'' is not valid, because we did not only determine PCI in platelet lysates, but also used different methods to show PCI synthesis and uptake. We agree with Dr Meijers that even at the 28-fold higher concentration (as compared to the values published by Nishioka et al[2]) platelet PCI can hardly influence the concentration of PCI in plasma, but that was never postulated. This assumption is also supported by the observation that the concentrations of PCI in serum are not higher than in plasma (what would be expected if significant amounts of PCI were released from activated platelets). However, at sites of platelet accumulation, and especially if PCI is bound to stimulating substances (e.g. proteoglycans, phospholipids), platelets might contribute to an increase in the local PCI activity.

The remark concerning the concentration of active PCI in platelet lysates (''second point of concern'') is incorrect. We assume that Dr Meijers might have misread the respective paragraph in the ''Results'' section of our article. We determined active PCI antigen in platelet lysates, which were derived from a platelet suspension containing an approximately 10-fold higher platelet concentration (2×10^9 platelets/mL) than blood. These platelet lysates (which had a PCI antigen concentration of 160 ng/mL) contained 14% (=812 ng/mL) of the active PCI antigen present in plasma (5.8 µg/mL). Therefore, the specific activity of platelet PCI is not 50-fold, but only 5-fold higher than the specific activity of plasma PCI. This is stated in our article in ''Results'' as well as in the ''Discussion'' sections. We have furthermore discussed some possible explanations for the higher specific activity of platelet PCI as compared to plasma PCI, such as the possibility that platelet PCI might be bound to substances stimulating its activity (eg, proteoglycans). This is not unlikely, since we have shown previously that cellular proteoglycans can stimulate the interaction of PCI with urokinase.[3] On the other hand, the active PCI antigen assay used in the present report is also based on the interaction of PCI with urokinase. However, we apologize if our data were not presented clearly enough. We hope that it is now clear that our antibody detects PCI and not other antigens, and we therefore cannot understand the last sentence of the second paragraph of Dr Meijers' letter, on the use of this antibody, which was published several years ago.[4]

As far as reverse transcription polymerase chain reaction (RT-PCR) is concerned, we are of course aware that the problem of contamination of platelet preparations with leukocytes can never be completely ruled out. However, this is stated in our article and we have therefore also confirmed PCI-synthesis in megakaryocytes by in situ hybridization of bone marrow sections.[1]

We completely agree that, in fact, the physiological role of PCI is not yet known. We therefore also hope that transgenic mice will help clarifying the role of PCI in hemostasis and in other systems. In fact, PCI (−/−) mice recently generated by Dr P. Uhrin from our department don't show any obvious disturbances of the hemostatic system (unpublished observations).

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