Correspondence

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The accompanying letter of Dr. Girolami was received in early March 1986, and Dr. Talstad's answer in April 1986.

The letter of Dr. Girolami suggests that as the discoverer of Pivka I was biased in favor of articles that stress its importance. This made me decide to try and separate the editorial and scientific responsibilities. I therefore asked a main editor to act as managing editor in this matter. I apologize to Dr. Girolami, to Dr. Talstad and to the reader for the delayed publication of these letters. I decided to reassume my editorial responsibilities in this matter and publish the letters as they were originally received.

Prof. Dr. H.C. Hemker

Comments on the Significance of Differences between Thrombotest and Normotest in the Study of Coumarin Plasma

Dear Sirs,

I have read with interest and great dismay at the same time the two papers by Dr. Talstad on prothrombin time [1, 2]. The two papers contain in fact an endless number of pitfalls, mistakes, wrong statements and unjustified claims which can be summarized along the following lines: (1) The aliquot of the reagents used in the assay system is reported to be 0.2 ml [p. 304] in one paper and 0.25 ml in the other paper [p. 311]. (2) The plasma shows cold activation (centrifuged at 4°C for 20 min). This may influence the test unless whole blood (WB) is treated in the same way. (3) In figure 2 [p. 306], on the left, no difference is noted between the two thromboplastins with 25 µl. The normotest (NT) 50 µl is not plotted. I wonder why. The figure is an example of manipulation of results. (4) No statistical analysis of the curves is presented. No standard deviations are supplied. From approximate calculations it seems to us that the changes observed might not be significant. (5) No data are supplied for 'non-warfarin' patients. Are they liver cirrhosis patients? What kind of cirrhosis? (6) Figure 1 [p. 305] is unintelligible. For example: there are two keys, but three curves. (7) Figure 4 [p. 308], the comparison between 25 µl whole blood normotest and 50 µl whole blood Thrombo-test is untenable. The results obtained with

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the same aliquots should have been compared. (8) Figures 3 and 4 [p. 314f.] are not comparable. The legends of the figures are the same but not the contents. The same aliquots should be compared in both groups of patients. (9) Table 4 [p. 316] indicates that clear differences exist between WB and plasma both in the Thrombotest (TT) and NT system. The comment 'Series

from different groups of patients' is pathetic! (10) The effect of RBC on the test has not been evaluated. Liver disease may cause RBC abnormalities, coumarin patients are supposed to have normal RBC. (11) Figure 2 [p. 313] is not correct. In the right lower quadrant, the points corresponding to 0.7 PCV are probably misplaced. If these points were correctly placed, a curve similar to the one observed with TT could have been obtained. (12) At the bottom of page 307 a sentence like this 'The ratios between TT and NT in pooled normal plasma showed a markedly different slope, obviously caused by the PIVKA inhibitor'. From such a sentence one can't help gathering the impression that even normal plasma contains an inhibitor! (13) From table 1 [p. 307], it seems that method A (WB) remains practically unchanged with the exception of TT 10 μ l. On the contrary, method B (plasma) varies considerably in every instance. This is probably due to cold activation and/or to the smaller amount of citrate per milliliter present at lower artificial PCV. It is absolutely wrong to claim that 'the difference between method A and B ... increased with increasing sample size'. Quite the contrary may be true. It may be seen, in fact, that 10 μ l TT shows a larger difference (about 4.5%) than TT 25 μ l or TT 50 μ l. It is true that NT 25 μ l has a much larger difference than NT 10 μ l, but this cannot be maintained to have any significance, since NT is considered by the author to have little if any sensitivity to inhibitors! This is a crucial point, since it is maintained to indicate the presence of inhibitors. Furthermore, it is not clear what method A at 0% artificial PCV means. (14) In figure 4b [p. 308] the same aliquots (25 or 50 μ l) of TT and NT should be compared. Incidentally, in figure 4a [p. 308], at least 30 patients (dots) show ratios of less than 2. What kind of stable anticoagulation is that? Proposed and accepted TT ratios for proper anticoagulation are at least 3-4! (15) The observation that factor X levels were higher than the TT or NT percentile figures confirms our findings, namely that global tests, in warfarin-treated patients, are lower than single-factor assays. (16) Finally, it is surprising to see that papers which do not support the inhibitory theory have not been quoted. The biological effects of prefactors or PIVKAS remain to be proved, at best. The results observed, granted that they might have a significance, should have been surmised to indicate, perhaps, the existence of an inhibitor. To state that the changes observed indicate the presence is unjustified, if not wrong. This is the typical example of findings interpreted according to a preconceived theory.

The above-mentioned list of remarks is surely incomplete. It is shocking to see that none of these comments have been raised by the managing editor or by a reviewer. Incidentally, probably no reviewer has ever had the chance to look at the papers, since they were submitted on March 15 and accepted on March 25, 1985. Probably this is the 'acceptance' reserved to papers supporting the inhibitory theory. This is one of the privileges of a managing editor, but of course also one of his responsibilities. Papers appear to

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be accepted not because they pursue the truth or because of their contents, but only because they support a preconceived and never proved theory. Is this a proper approach? In other words is this scientifically honest? – I leave the decision to the readers. I am sure that in the present form the two papers would not have been accepted by any serious journal! They needed, at least, extensive revision. Bias may play 'dirty tricks' sometimes. For their information I am sending a copy of this letter to other editors and to the publisher.

References

Talstad I: How does the Pivka inhibitor interfere with the one-stage prothrombin time? *Haemostasis* 1985;15:304-309.

Talstad I: An analysis of the one-stage prothrombin time. *Haemostasis* 1985;15:310-317.

Dr. Antonio Girolami

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Comments to Antonio Girolami

(1) The reagent volume used for TT was 0.25 ml. (2) Our routine procedure to collect blood on ice and centrifuge at +4°C was used. For TT and NT we have found no difference between our routine procedure and using room temperature; thus there is no cold activation. (3) The difference between TT and NT using 25-μl sample size was not significant for the PCV correction factors, which correct the coagulation activities towards those at PCV 0.45. However, there were significant differences between coagulation activities in the same series (table 1). I have only presented statistics between methods A and B. I agree that statistics might have been done between different assays: For instance between TT and NT at 25-μl sample size: At PCV = 0, TT = 14.3, NT = 21.7, $p < 0.001$. However, which assays should be compared and at which PCV? If compared against the commonly used reference assay TT with 50-μl sample size, the question asked between TT and NT at 25-μl sample sizes could not be answered. NT using 25-μl sample size is a universal assay, while NT and TT, using 10-μl sample sizes, did not cover low enough activities in warfarin-treated patients (table 1). Nobody applies 50 μl in NT assaying. (4) Has been explained in (3). (5) The 'non-warfarin' patients [fig. 1, p. 305] used in factor X assaying were randomly collected from patients not treated with warfarin. The liver disease () and warfarin () patients are marked. (6) Is explained in (5). (7) I do not agree. TT has been accepted as a 2nd International Standard, 50 μl whole blood is the commonly used reference assay in warfarin-monitoring, which can be compared with any other assay. (8) I do not agree. This is described in the text [p. 313] and repeated in (3) above. (9) The difference between methods A and B when using whole blood as test material was slight. Since many methods apply plasma instead of whole blood it was decided to compare methods A and B in

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whole blood and plasma in another series. (10) RBC causing different PCV interferes with the assays, not the shape changes of RBC. (11) The PCV correction curve A is theoretically calculated. The measured values differed from the theoretical curve in warfarin-treated patients due to the Pivka inhibitor. (12) The different slopes were obviously caused by the Pivka inhibitor. (13) No. Method A applies PCV correction factors [fig. 2, p. 312], also obtaining correction for the Pivka inhibitor. Method B corrects for the PCV mathematically, but does not correct for the Pivka inhibitor. PCV = 0 means plasma. (14) The therapeutic ranges for TT were 2.0-4.3 as marked in the figure. (15) These relations will be presented in more detail elsewhere [1]. (16) I greatly disagree. The present papers demonstrated the influence of the Pivka inhibitor at different

sample sizes at various PCV, at different thromboplastins and the absence of Pivka inhibitor in non-warfarin-treated patients. The Pivka inhibitor is the main reason why the standardization of one stage prothrombin assays has been and still is a great problem [2].

References

Talstad I: The influence of blood clotting factors on the one-stage prothrombin time (PT). Int Soc Haematol, Sidney 1986 (F-301-15).

Talstad I: The standardization of the one-stage prothrombin time. 21 st Int Soc Haematol, Sidney 1986 (F-301-9).

Announcement

Prize Biochemical Analysis 1990

Conference 'Biochemische Analytik', Munich, FRG, May 8-11, 1990

The German Society for Clinical Chemistry awards the prize Biochemical Analysis every 2 years at the Conference 'Biochemische Analytik'. The prize of DM 50,000.- is donated by Boehringer Mannheim GmbH for outstanding and novel work in the field of biochemical analysis or biochemical instrumentation or for significant contributions to the advancement in experimental biology, especially relating to clinical biochemistry. If several authors are involved in this work, please indicate the name(s) of the candidate(s).

Competitors for the prize 1990 should submit papers concerning one theme, either published or accepted for publication between October 1, 1987, and September 30, 1989, before October 15, 1989, to: Prof. H. Feldmann, Secretary of the prize Biochemical Analysis, Institut für Physiologische Chemie der Universität, Goethestrasse 33, D-8000 München 2 (FRG).