SIR,—In view of the inaccuracy of the clinical diagnosis of DVT or PE, it is surprising that only 83% of patients analysed in the British Thoracic Society's study underwent investigations to attempt to objectively confirm a clinical suspicion of venous thrombo-embolism before anticoagulation. Furthermore, objective confirmation of a clinical suspicion of failure or recurrence was sought in only 42% of patients. Although not an objective of the study, since 4·9% of patients had complications of anticoagulation (including one death) is it reasonable to initiate or prolong anticoagulation therapy on the basis of a clinical suspicion of venous thrombo-embolism without objective confirmation?

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Effect of thrombolytic treatment delay on myocardial infarct size

SIR,—Intravenous thrombolytic therapy is now given routinely in patients with acute myocardial infarction (AMI). In large trials survival seemed better in patients treated early, and many hospitals restrict therapy to those presenting within 6 h after first symptoms. However, the effect of treatment delay on survival is not pronounced and Dr White (July 25, p 221) has advocated extension of this time limit. Measurement of infarct size allows more detailed analysis of the effect of treatment delay in thrombolytic therapy, since quantitative estimates can easily be obtained in more than 95% of patients.

In three randomised trials of thrombolytic therapy[1-3] by the European Cooperative Study Group, infarct size was calculated as the cumulative activity of myocardial α-hydroxybutyrate dehydrogenase released per litre of plasma during the first 72 h after AMI (Q_{72}). 1374 patients with AMI were allocated to treatment with alteplase (single chain, Boehringer Ingelheim, Germany) within 6 h of first symptoms. Alteplase was given as a 10 mg bolus, followed by 50 mg infused over the first hour and 20 mg/h over the next 2 h. All patients received aspirin. Median infarct size (95% CI) calculated in 1334 patients (97%), was 666 U/l (591–736).

The figure shows the effect of treatment delay on infarct size. Overall linear regression analysis demonstrated a significant ($p < 0.001$) effect of treatment delay on infarct size: $Q_{72} = 584 + 65$ (treatment delay) U/l. However, additional benefit of very early treatment is apparent and deviation of linearity was significant. In 61 patients treated within 75 min, median infarct size was 331 (258–602) U/l and, within this group, no longer related to delay in treatment. In the remaining 1272 patients, a linear relation persisted: $Q_{72} = 685 + 56$ (treatment delay) U/l ($p = 0.0001$). In 353 placebo-treated patients,[1] median infarct size was 867 (779–933)

Median infarct size

Q_{72} (U/litre)
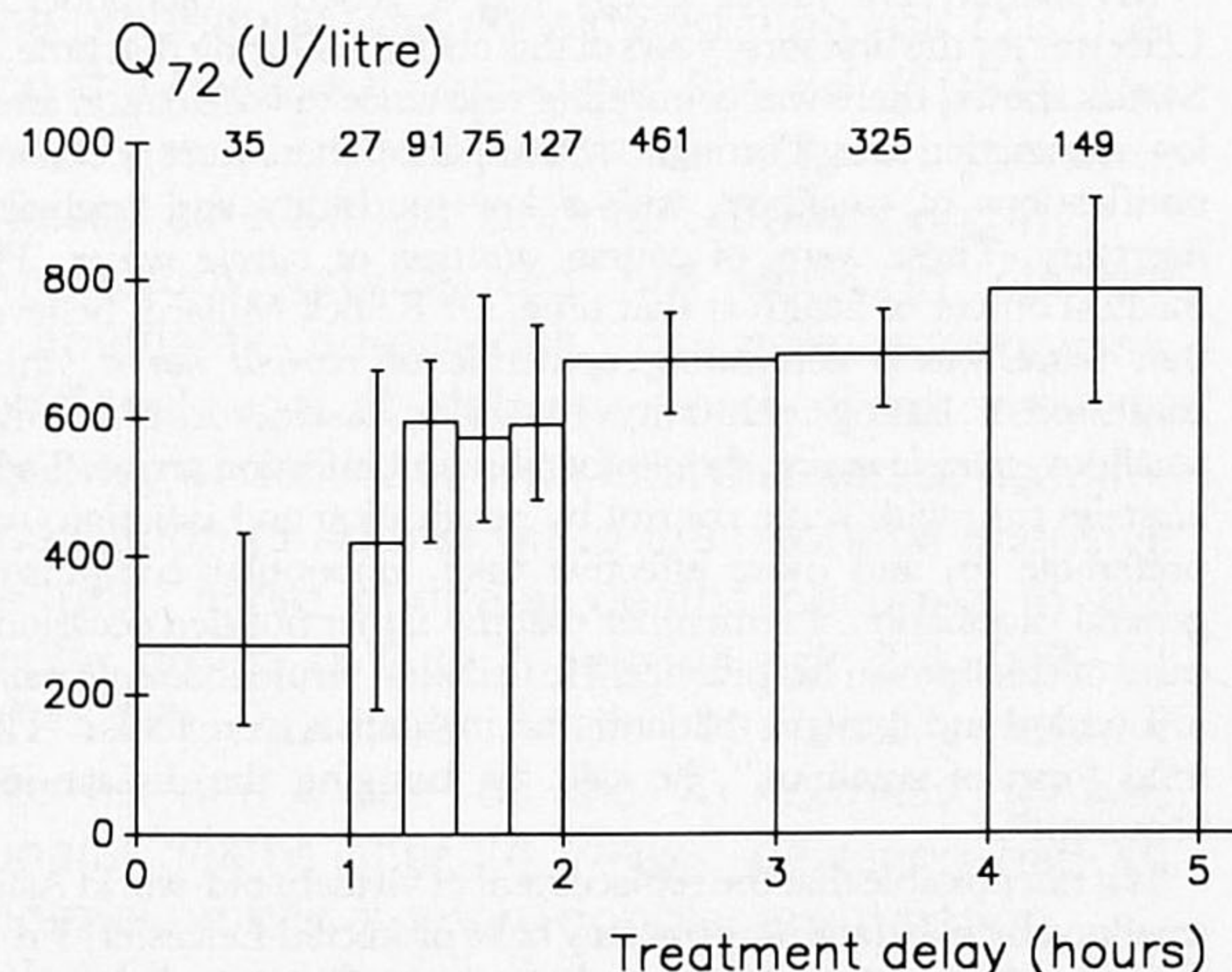
Effect of delay of thrombolytic treatment on infarct size.

No of patients shown at top of each column. Columns = means, vertical bars = 95% CI.

U/l, and no effect of treatment delay on infarct size was recorded.

Thus, very early treatment with intravenous alteplase results in a substantial (30–70%) reduction of infarct size. More than half this effect is lost when treatment is delayed by more than 60–75 min. These results emphasise the importance of finding strategies for pre-hospital thrombolysis.[4]

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1. Van der Werf F, Arnold AER, for the ECSG. Intravenous tissue plasminogen activator and size of infarct, left ventricular function and survival in acute myocardial infarction. BMJ 1988; 297: 1374–79.
2. Simoons ML, Arnold AER, Betriu A, et al. Thrombolysis with tissue plasminogen activator in acute myocardial infarction: no additional benefit from immediate percutaneous coronary angioplasty. Lancet 1988; i: 197–203.
3. De Bono DP, Simoons ML, Tijssen J, et al. Effect of early intravenous heparin on coronary patency, infarct size, and bleeding complications after alteplase thrombolysis: results of a randomised double blind ECSG trial. Br Heart J 1992; 67: 122–28.
4. Bouten MJM, Simoons ML. Strategies for pre-hospital thrombolysis: an overview. Eur Heart J 1991; 12 (suppl G): 39–42.

Reverse passive haemagglutination test for identification and serotyping of polioviruses

SIR,—The identification of poliovirus by the neutralisation test is laborious, so we have developed a simple and rapid reverse passive haemagglutination (RPHA) test.

40 stool specimens were obtained from immunised infants 1 week after oral poliomyelitis vaccination. Polioviruses were isolated on Vero cells and identified by standard methods.[1] Mouse monoclonal antibodies Mah4e (anti-poliovirus type 1), S2–16 (anti-poliovirus 2), and SuWa (anti-poliovirus 3) were used to coat sheep red blood cells (SRBC) (SRBC-Mah4e, SRBC-S3–16, and SRBC-SuWa).[2] SRBC were fixed with 0·5% glutaraldehyde at room temperature for 1 h and washed with phosphate-buffered saline (PBS). The fixed SRBC were treated with tannic acid (30 μl/ml) at 37°C for 10 min. After washing with saline, a 6·0% suspension of the tanninised SRBC was prepared in McIlvain buffer containing 0·5 mol/l NaCl (pH 5·0) and mixed with an equal volume of poliovirus monoclonal antibody which was purified by 33·3% saturated ammonium sulphate (50 μg/ml) from immunised BALB/c mouse ascites with antibody-producing hybridoma. The mixture was incubated at room temperature for 1 h to prepare antibody-sensitised SRBC. The SRBC was washed with PBS, and a 0·6% suspension was prepared in PBS containing 2·0% heat-inactivated rabbit serum (PBS-RS). The monoclonal antibodies reacted with both vaccine and wild strains of homologous serotype by enzyme-linked immunosorbent assay.

The RPHA test was by microtitration on plates with 120 V-shaped wells. Culture fluid of inoculated stool samples that were cytopathic on Vero cells were used as specimens for the RPHA test. Serial two-fold dilutions of the specimens were made in triplicate with a 25 μl loop and a diluent of PBS-RS containing 1·0% SRBC. SRBC-Mah4e, SRBC-S2-16, and SRBC-SuWa were added to the 1st, 2nd, and 3rd dilution series, respectively. After shaking, the microtitration plate was covered and kept at room temperature for 1 h, and agglutination (poliovirus positive) was observed by eye.

The sensitivity of the RPHA test for poliovirus was estimated by the tissue-culture infective dose$_{50}$ ($TCID_{50}$). SRBC coated with type-specific monoclonal antibody caused agglutination with the homologous poliovirus only. $TCID_{50}$ per 50 μl was $10^{6.04}$ for SRBC-Mah4e, $10^{5.61}$ for SRBC-S2-16, and $10^{5.85}$ for SRBC-SuWa. Thus RPHA is sensitive for poliovirus detection. No false negative or positive reactions occurred:

Neutralisation type	No of samples	RPHA typing
1	3	3
2	29	29
3	8	8

Although the numbers examined were small, the RPHA test was useful and practicable for the identification of poliovirus. SRBC coated with type-specific monoclonal antibody gave no non-specific