Technical method

Histological demonstration of platelet thrombi with a double silver impregnation argyrophil stain

DN SLATER, G ANDERSON, JF MARTIN *Departments of Histopathology and Medicine, Royal Hallamshire Hospital, Sheffield*

Thrombi vary in composition with differing percentages of fibrin, platelets, and red blood cells. Of these components, only fibrin is routinely demonstrated by histological staining techniques. We describe the use of a double silver impregnation argyrophil stain to demonstrate platelet thrombi.

Case report

A 74-year-old retired metal worker presented with gangrene in the left 1st, 2nd, and 3rd toes and the left 4th finger. The platelet count was normal ($210 \times 10^9/l$) but spontaneous platelet aggregation was present on aggregometry¹; the platelet aggregate ratio was low (0.45) using the method of Wu and Hoake;² the presence of circulating platelet aggregates was confirmed by the inspection of a peripheral blood film and the β -thromboglobulin concentration was raised (83 ng/ml). These results were considered indicative of intravascular platelet aggregation. The peripheral blood count and other coagulation tests were normal. Cardiac ultrasound examination was normal.

HISTOLOGICAL STUDIES

Light microscopy of the digital vessels in non-gangrenous areas showed luminal occlusion by faintly eosinophilic granular material. This stained negatively for fibrin by both Martius Scarlet Red and phosphotungstic acid haematoxylin.

Examination of the vessels by electron microscopy (Philips EM400) showed that the material consisted of platelet thrombus (Fig. 1) with minimal amounts of fibrin. An interesting feature was the presence of scattered granules in the cytoplasm of many platelets. The presence of electron-dense granules in unstained sections and the identification of calcium with x-ray microanalysis (EDAX system with EPIC computer) indicated that the granules included dense bodies.

Accepted for publication 13 April 1982

Fig. 1 Thrombus with partially granulated platelets. $\times 12\,500$.

As platelet granules are known to contain various amines, including 5-hydroxytryptamine, histamine, and catecholamines, it was considered possible that the granules may be demonstrable by stains normally used for APUD granules. Consequently, paraffin sections of the vessels were stained by the diazo reaction and Masson-Fontana for argentaflin granules and by a modification (as below) of Pascual's double silver impregnation technique for argyrophil granules.³ Negative staining was obtained with the argentaflin stains but strongly positive granular staining was revealed with silver impregnation method (Fig. 2).

Platelet-rich plasma was prepared from whole blood by centrifugation at 140 g for 10 min. When this was stirred in an aggregometer, aggregation was seen to occur. Ultrastructural examination of these aggregates showed central platelets containing dense bodies and alpha granules; the peripheral platelets were degranulated (Fig. 3). Light microscopy of aggregates from the same aggregometer cuvette showed positive argyrophil staining in the centre of the aggregates.

Material and methods

MODIFICATION OF PASCUAL'S SILVER IMPREGNATION METHOD

- 1 Take sections to water and rinse well in distilled water.
- 2 Treat in preheated 5% silver nitrate at 60°C for 45 min.
- 3 Rinse briefly in distilled water.

Fig. 2 Digital vessel with thrombus. Positive argyrophil staining with modified Pascual stain. $\times 240$.

Fig. 3 Platelet aggregates with granules. $\times 5000$.

- 4 Reduce in preheated Bodian's reducer (hydroquinone 1g; sodium sulphite crystals 10g; distilled water 100 ml) at 60°C for 5 min.
- 5 Wash well in tap water; rinse in distilled water.
- 6 Treat sections for a further 20 min in preheated 5% silver nitrate at 60°C.
- 7 Rinse briefly in distilled water.
- 8 Reduce as in step 4.
- 9 Wash well in tap water.
- 10 Tone in 1:10 000 gold chloride in distilled water for 10 min.
- 11 Wash in water.
- 12 Fix in 5% sodium thiosulphate (hypo) for 2 min.
- 13 Wash well in water.
- 14 Stain nuclei in nuclear fast red for 3 min (nuclear red 0.1g; aluminium sulphate 5g; distilled water 100ml; *Method*: add nuclear fast red and aluminium sulphate to the distilled water, boil for 3 min, cool and filter).
- 15 Rinse in water, dehydrate, clear and mount in resin.

Results

Argyrophil granules – black

Nuclei – red.

Discussion

The positive argyrophil reaction in this case correlated with the presence of granulated platelets in the thrombi. The latter finding was unexpected as it indicated that the platelets had undergone aggregation but not secretion.⁴ Ultrastructural examination of thrombi in other diseases has shown different degrees of platelet degranulation and a corresponding variability in the success of argyrophil positivity. However, consistently positive results have been observed in other cases with thrombi associated with spontaneous platelet aggregation and circulating platelet aggregates. Thrombi containing largely fibrin give a negative reaction indicating that the positive result was not due to non-specific silver precipitation.

The exact cause of the positive argyrophil reaction remains uncertain. However, the negative argentaffin reaction indicates that it is due to amines other than 5-hydroxytryptamine.

Although platelet function can be tested in vitro by both mechanical and biochemical methods, techniques for measuring in vivo platelet function are too invasive to be used in man.⁵ There is much indirect evidence that platelets are causally involved in arterial thrombosis and the staining method described here is a histological tool that can demonstrate platelets to be a cause of vascular occlusion.

References

- ¹ Vreken J, Van Aken WG. Spontaneous aggregation of blood platelets as a cause of idiopathic thrombosis and recurrent painful toes and fingers. *Lancet* 1971;ii:1394-7.
- ² Wu KK, Hoake JC. A new method for the quantitative detection of platelet aggregates in patients with arterial insufficiency. *Lancet* 1974;ii:924-6.
- ³ Pascual JSF. A new method for easy demonstration of argyrophil cells. *Stain Technol* 1976;51:231-5.
- ⁴ Martin JF, Holland TK, Russell RGG. Evidence for the involvement of microtubules in the ADP-induced secretion of platelet ¹⁴C-S-hydroxytryptamine. *Thromb Res* 1981;22:525-33.
- ⁵ Bee D, Leach E, Martin JF, Suggett AJ. The effects of vincristine on platelet aggregation studied by a filter loop technique in the rat. *Br J Pharmacol* 1980;69:227-31.

Requests for reprints to: Dr DN Slater, Floor E Pathology, Royal Hallamshire Hospital, Sheffield S10 2RX, England.

Letters to the Editor

Word and data processing in histopathology laboratories

As judged by the number of visitors and enquiries about the system in our laboratory, there is a considerable interest around the country in word and data processing facilities for histopathology laboratories. Accounts of our system have appeared elsewhere^{1,2} but we felt that many potential users of such systems might be interested in the sorts of problems which will almost inevitably arise when a computer system is installed and developed.

Installation

There were initial problems over the wiring from the computer to the various terminals. The Department of Medical Physics offered to do this, but the hospital administration felt that this was politically unwise in view of possible union objection. In consequence the hospital Works Department performed the job, which was done admirably, but four weeks after the Department of Medical Physics could have completed it.

The instruction manuals and system documentation accompanying computers and commercial software are seldom complete or written in a concise, comprehensible form. Our word processing manual was, in places, particularly bad and took a lot of understanding. The suppliers now have a much improved version.

Personnel

Many people have, if not open hostility to computer systems, then a suspicion of them. Careful public relation exercises may be needed to overcome such feelings. Our secretarial staff settled in quickly to the system and have been granted salary increases because of their new responsibilities. The negotiations revolving around this particular aspect were quite lengthy.

Hardware

Computers are only machines and, as such, will break down from time to time. Our computer has been out of action for about one week in the year or so of use. Some protocol has to be established for when the machine breaks down in order to ensure that the services of the laboratory are not interrupted. Various service contracts are available from computer manufacturers. These vary in price depending on the service required; minor problems are sometimes soluble by the scientific staff, but formal contracts are vital. Printers are worked very hard and, partly for this reason, it was decided to duplicate ours. This not only gave a back-up, but allowed far greater flexibility in printing on different types of paper—for example, histology and post-mortem reports.

Software

Software problems are the main headache in any computer development. The com-

mercially purchased programs are never totally error-free. Of course when one is originally looking around the market for what is available, salesmen are not at pains to point these out. These errors, or bugs, must be overcome by the scientific staff. This staff cannot be expected to have grasped every last limitation of the programs before purchase, just as the clinical staff cannot be expected to have totally realised what they require of the system. These two groups of people must be in constant and close collaboration as the system is developed so that the laboratory actually ends up with a system which is good both in the computer scientist's eyes and in the pathologist's eyes. This collaboration is crucial and is why we believe that a good system cannot be developed quickly if the scientific staff are not resident in the hospital. This close liaison has allowed the development of a sophisticated set of coding, data storage, and archiving programs. These, as indicated above, were not devoid of developmental problems. A very simple example is shown in the fact that although 1981 was used as a developmental year, all the histological programs were running, and error free, in mid-November, 10 months after the installation of the equipment. Efforts were then concentrated on other programs, only to realise, just after Christmas, that the laboratory specimen numbering format of XXXX81 would produce problems at the change over of the