Immunohistochemical identification of erythroid precursors in paraffin embedded bone marrow sections: spectrin is a superior marker to glycophorin

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Abstract

Aim—To investigate whether spectrin can be used as an immunohistochemical marker for erythroid precursors in routinely processed paraffin embedded bone marrow sections.

Methods—Bone marrow biopsies and clot sections were stained with rabbit anti-human erythrocyte spectrin antibodies, specific for erythroid cells as shown by western blotting and bone marrow smears, and compared to sections stained with antiglycophorin monoclonal antibodies (JC159 and Ret49f).

Results—Antispectrin antibodies resulted in diffuse cytoplasmic staining of early erythroblasts and membranous staining of late erythroblasts as well as erythrocytes. In haematopathological samples, immature erythroid cell clusters were clearly identified. In contrast, antiglycophorin monoclonal antibodies resulted in only membranous staining of late erythroblasts, and faint staining of early erythroblasts.

Conclusions—Spectrin may be a superior marker to glycophorin for the identification of erythroid precursors in paraffin embedded sections.

Keywords: spectrin, erythroblast, immunohistochemistry

In paraffin embedded tissue, immunohistochemistry is useful to identify erythroblasts, as erythroid precursors, proerythroblasts, and basophilic erythroblasts are not easily differentiated from other type of precursors. So far, antibodies against haemoglobin, glycophorin A, and glycophorin C have been used to identify erythroid cells in paraffin sections. However, the ability of these antibodies to identify erythroid precursors has not been determined.

The expression of erythroid membrane cytoskeleton components—spectrin, glycophorin, and band 3 protein—during mammalian and human erythroid cell differentiation has been extensively studied. While spectrin is a cytoskeleton component seen from early to terminal erythrocyte differentiation, the usefulness of this protein as a diagnostic marker has not been tested. In the present study, we used anti-human erythrocyte spectrin antibodies to assess routinely processed paraffin embedded sections.
washed in phosphate buffered saline (PBS) and allowed to react with peroxidase-Envision (Dako) for 30 minutes. The sections were developed with diaminobenzidine and counterstained with haematoxylin. Negative controls were treated in the same manner, but without the primary antibodies.

**Results**

**REACTIVITY OF THE ANTISPECTRIN AND ANTIGLYCOPHORIN ANTIBODIES WITH NORMAL ERYTHROID CELLS AT DIFFERENT MATURATION STAGE**

In western blotting of bone marrow cell lysates, antihuman erythrocyte spectrin antibodies formed two bands, corresponding to α and β spectrin. In sections of adult tissues, the antibodies only reacted with erythroid cells at all stages of maturation in bone marrow, with extramedullary haematopoietic foci in spleen and liver, and with blood erythrocytes. Myeloid cells and megakaryocytes were not stained. Antigen retrieval with microwave treatment was essential to obtain good staining results. Immunohistochemical staining is influenced by decalcification procedures, with intensity markedly decreasing in decalcified bone marrow necropsy samples. In control cases, the antispectrin antibodies stained early erythroblasts, forming clusters and large isolated erythroblasts (fig 1A). Both these cell types had diffusely stained cytoplasm. Late erythroblasts, and erythrocytes, had stained membranes (fig 1B). JC159 (antiglycophorin A) and Ret49f (antiglycophorin C) weakly stained membrane of early erythroblasts, forming clusters. The membranes of late erythroblasts, as well as erythrocytes, were all strongly labelled.

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**Figure 1** Immunoperoxidase staining with antispectrin in the bone marrow. A cluster of early erythroblasts (A) and a cluster of late erythroblasts (B). Spectrin is stained in the cytoplasm of early erythroblasts and on membranes of late erythroblasts.

**Figure 2** Immunoperoxidase staining of clusters of erythroid precursors with antispectrin antibodies (A) and JC159 (B) in serial sections from a patient with myelodysplastic syndrome. This sample shows erythroid hyperplasia. Spectrin staining is seen mainly in early erythroblasts. Late erythroblasts and erythrocytes are heavily stained with JC159, but early erythroblasts are faintly stained.

**Figure 3** Cytoplasmic staining of spectrin in bone marrow giant proerythroblasts from a parvovirus B19 infection patient (A). JC159 failed to stain the giant proerythroblasts (B, arrow).
Immunohistochemical identification of erythroid precursors

In this study, we performed paraffin embedded immunohistochemistry using antispectrin antibodies. Intracellular haemoglobin has been reported to be a sensitive and specific marker for erythroid precursors in paraffin embedded sections. However, the sensitivity of this marker for early erythroid precursors is questionable because haemoglobin can be detected during the late stage of erythroid cell differentiation. Nowadays, antiglycophorin A and antiglycophorin C monoclonal antibodies are widely used instead of antihaemoglobin antibodies, and Erber et al showed that antiglycophorin A monoclonal antibody JC159 was superior to antiglycophorin C monoclonal antibody Ret49f for the staining of blast cells of erythroleukaemia in paraffin sections.

We found that staining patterns and intensities of erythroblasts were different between antispectrin and antiglycophorin monoclonal antibodies. Consistent with previous reports on immunocytochemistry in bone marrow smear, antispectrin antibodies showed strong cytoplasmic staining, whereas antiglycophorin monoclonal antibody showed membranous staining. Although glycophorin has been found to be expressed by erythroid cells from the morphologically recognisable erythroblasts arising just after the erythroid colony forming unit (CFU-E) stage to mature erythrocytes, glycophorin expression was weak in early erythroblasts in routinely processed paraffin embedded sections. This may be because the detection sensitivity of paraffin section immunohistochemistry is lower than that of flow cytometry. Antispectrin antibody staining in smears of bone marrow cells was shown to be localised in the cytoplasm of late erythroid burst forming unit (BFU-E) in which band 3

In conclusion, antispectrin antibodies are more useful for detecting erythroid precursors in paraffin embedded immunostaining than antiglycophorin monoclonal antibodies (JC159 and Ret49f).