A quantitative study of eosinophil polymorphs in Hodgkin's disease

WJ Fuggle, J Crocker, PJ Smith

From the Department of Histopathology, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST

Summary

Eosinophil polymorphonuclear leucocytes (polymorphs) were counted in 45 specimens from patients with Hodgkin’s disease and five specimens from patients with reactive follicular hyperplasia. The use of chlorazol fast pink BK, a little known stain for eosinophil polymorphs, combined with image analysis facilitated rapid and reliable counting. Significant differences were found between the mean percentages of eosinophil polymorphs in the Rye subtypes of Hodgkin’s disease. The numbers of eosinophil polymorphs in specimens from patients with reactive follicular hyperplasia were very low and could not be counted.

The tissue of Hodgkin’s disease infiltrates is highly polymorphic, containing a wide range of cell types including lymphocytes and macrophages together with epithelioid, Hodgkin, and Reed-Sternberg cells. In addition, eosinophil polymorphonuclear leucocytes (polymorphs) are often present in variable numbers. There is some subjective evidence that the content of eosinophil polymorphs may differ with the Rye subtype of Hodgkin’s disease, and in one study it was suggested that eosinophil polymorphs were more common in biopsy specimens showing fibrosis. In order to obtain objective data, we have examined a series of specimens from patients with Hodgkin’s disease and have expressed the number of eosinophil polymorphs present as a percentage of all cells present. This has been facilitated by means of a dye which intensely stains eosinophil granules—namely, chlorazol fast pink.

Material and methods

Patients and tissues

All of the cases were taken from the files of East Birmingham Hospital over 12 years. They were classified according to the Rye convention by one of us (JC).

Forty five specimens from 38 patients with Hodgkin's disease were examined. These comprised 15 nodes showing lymphocyte predominance, nine of the nodular sclerosing variety, 14 specimens of mixed cellularity, and seven with lymphocyte depletion. One specimen appeared to show progression from lymphocyte predominance to the nodular sclerosing variety. Five cervical lymph nodes from patients with reactive follicular hyperplasia were also studied. The ages and sexes of the patients concerned are shown in Table 1.

Fixation and staining

Specimens were cut into thin slices, fixed at room temperature in 10% formol-saline for 24–48 h, and routinely processed to paraffin wax. Sections were cut at 4 μm thickness and then deparaffinised and stained in 1% chlorazol fast pink BK (BDH Chemicals Ltd, Gurr, Atherstone, Warwickshire, England) in 50% ethanol for 30 min at room temperature, as described by Li et al. Mayer’s haemalum was substituted for methyl green as a counterstain. Subsequently, the sections were dehydrated through graded alcohol concentrations, passed through xylene, and mounted in balsam.

Counting procedure

Counting of cells was greatly facilitated by means of an interactive user controlled image analyser, the Reichert-Jung (Kontron) “Digiplan”. This system is closely related to the Reichert-Jung (Kontron) MOP series of analysers, previously described in detail. An image of the section under study was thrown onto the graphic tablet of the Digiplan by means of a projection microscope, using a 40× objective and 1:25× projection lens. A simple grid overlay was used on the digitiser tablet to prevent recounting of cells, and a green filter was placed in
the system to enhance cell contrast. Fields were selected at random as done previously. All eosinophils in each field were counted and stored on one channel of the microprocessor, and then all nucleate cells were similarly counted and retained in another channel. About 2000 cells were counted for each specimen, and the numbers of eosinophils were expressed as a percentage of this total count. In the nodular sclerosis subtype, both fibrous and more cellular areas were included in the counting.

**Results**

In some cases more than one biopsy specimen was taken from a patient and these are indicated in Table 1, together with the sites from which tissues were removed. In one case, the Rye subtype in a patient appeared to have progressed from lymphocyte predominant to nodular sclerosing disease.

![Fig. 1 shows the scatter of percentages of eosinophil polymorphs in all specimens. Eosinophil polymorphs were absent in all of the nodes from patients with reactive follicular hyperplasia. The lymphocyte predominant specimens possessed 0 to 0-9% eosinophil polymorphs (mean 0-24%); the nodular sclerosing nodes 0 to 34-2% (mean 13-2%); those with mixed cellularity 0 to 20-8% (mean 5-35%); and those showing lymphocyte depletion...](http://jcp.bmj.com/article-pdf/37/3/267/30107482/jcp-37-3-267)
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Fig. 1  Mean percentages of eosinophil polymorphs in specimens from patients with Hodgkin's disease (HD) and reactive follicular hyperplasia (RFH). LP = lymphocyte predominant; NS = nodular sclerosing; MC = mixed cellularity; LD = lymphocyte depleted. Vertical bars show mean of range.

contained 8.2 to 31.9% eosinophil polymorphs (mean 22.6%).

These data, analysed by Student's t test, show significant differences between the various Rye subtypes (Table 2). There was no significant difference, however, between the reactive follicular hyperplasia and lymphocyte predominant Hodgkin's disease specimens.

Table 2  Significance of differences between Rye subtypes of specimens from patients with Hodgkin's disease and reactive follicular hyperplasia

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Fig. 2 Numerous darkly stained eosinophil polymorphs in a cellular nodule of nodular sclerosing Hodgkin's disease. Chlorozol fast pink stain with haemalum counterstain. Magnification ×180.

Discussion

Hodgkin's disease presents a highly polymorphic cellular picture with varying numbers of different cell types. The Rye convention classification depends on the subjective assessment of the relative proportions of these and any associated fibrosis to separate Hodgkin's disease into various subtypes, which have prognostic importance.1-4 Few morphometric objective studies have been performed on specimens from patients with Hodgkin's disease,3, 9, 11, 12 and these support the Rye classification only in broad terms.

Livesey et al11 performed differential cell counts on nine lymph nodes from patients with Hodgkin's disease using an eyepiece graticule and coverslip marking or clear film grid on the glass slide. They found an implied misnomer in the terminology of the Rye classification, with a higher percentage of lymphocytes in lymphocyte depleted than in mixed cellularity subtypes. They did not distinguish between eosinophil polymorphs and neutrophil polymorphs, counting them as a single category. This totally manual counting technique is extremely laborious as well as labour intensive. The study of Livesey et al is important because it was, to our
knowledge, the first recorded morphometric study of Hodgkin's disease.

Toth et al. performed eosinophil polymorph 
counts on a highly selected group of patients with 
Hodgkin's disease and found a percentage of eosinophil 
polymorphs of 50% or more to be 
associated with a poor prognosis and fibrosis. Their cell 
counting methods were not described.

Crocker et al. performed a quantitative study of 
α-naphthyl acetate esterase positive cells in 32 
specimens from patients with Hodgkin's disease 
using a similar method to the present study. The cell 
counts were performed using a graphic tablet linked 
to a multichannel interactive image analyser (Kontron MOP AMO) and camera lucida arrangement. 
α-naphthyl acetate esterase is a lysosomal enzyme 
which is a convenient marker for macrophages. Fewer macrophage type cells were found in mixed 
cellularity than in nodular sclerosing or lymphocyte 
depleted subtypes using this histochemical technique. The previous study by Livesey et al. had 
identified the highest percentage of non-neoplastic 
mononuclear cells in mixed cellularity on purely 
morphological grounds. The findings of Crocker et al. were subsequently confirmed using acid 
phosphatase as a macrophage marker.

Eosinophil polymorphs are relatively easy to distinguish in tissue sections even under low power light microscopy, and their tendency to cluster may lead to misleading impressions of their numbers. The chlorazol fast pink stain, which we used in this

study, has been described previously by Japanese 
workers but appears to be little used in the UK. The 
intensity of the staining response enabled the rapid 
and accurate enumeration of eosinophil polymorphs 
using the Reichert-Jung (Kontron) "Digiplan".

The results confirm, in broad terms, subjective 
impressions of numbers of eosinophil polymorphs in 
Rye subtypes reported by others. There is a distinct 
difference in eosinophil percentage in the lymphocyte predominant type compared with the nodular 
sclerosing, mixed cellularity, and lymphocyte 
depleted varieties. Specimens from patients with 
reactive follicular hyperplasia contain no countable 
eosinophil polymorphs. Our study also shows a 
significant difference between the numbers of eosinophil polymorphs in the four main subtypes of 
Hodgkin's disease, although there is overlap between 
groups. There is also variation between different 
samples from the same patient (Table 1). It has 
been suggested that the percentage of eosinophil 
polymorphs is related to age, but we have not found 
this to be true in our 45 samples. Neither have we 
found a higher percentage of eosinophil polymorphs 
in specimens from patients with fibrosis, as previously reported. The biological importance of these 
differences is unclear and it is interesting to note 
that the percentage of eosinophil polymorphs does not 
exactly mirror prognostic groups, there being a higher 
mean percentage of eosinophil polymorphs in 
the nodular sclerosing than in the mixed cellularity subtype. This was also true of α-naphthyl acetate 
esterase positive cells in a previous study.

The role of eosinophil polymorphs in Hodgkin's 
disease has not been elaborated, although they are 
often found in areas of necrosis. We have not seen 
this on chlorazol fast pink staining. Eosinophil 
polymorphs are produced in the bone marrow and 
pass into the tissues after a brief sojourn in the blood 
stream. They phagocytose particulate matter and 
have a higher concentration of peroxidase than 
neutrophil polymorphs. In common with the latter 
they are attracted by chemotactic lymphokines, which are, in part at least, released by transformed lymphocytes. It is possible that eosinophil polymorphs are attracted to Hodgkin's disease tissue as a result of the well recognised interaction between, for example, Reed-Sternberg cells and 
lymphocytes.

As stated by Taylor, it seems possible that the 
one popular Gordon biological test for Hodgkin's disease owed much of its success to eosinophil polymorphs within the sample. In this test suspensions of tissue from patients with Hodgkin's disease were injected intracerebrally and intravenously into rabbits with resulting neurological sequelae with positive samples.
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In view of previous studies of inter-observer disagreement in the classification of Hodgkin's disease, it is apparent that further morphometric studies of this condition may afford valuable data.

We are grateful to Mrs Pamela Jackson for secretarial assistance and graphics.

References


Requests for reprints to: Dr J Crocker, Consultant Histopathologist, Department of Histopathology, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST, England.