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*Original Papers*

# Assessment of Host Defence against Infection during Chemotherapy of Hodgkin's Disease

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***Summary.*** *Simultaneous measurements of leucocyte numbers and function have been made during combination chemotherapy for advanced Hodgkin's disease. There was a marked fall in the numbers of circulating mononuclear cells during the chemotherapy cycle in many patients. The migratory, phagocytic, candidacidal, and bactericidal activities of polymorphs and monocytes were frequently depressed. Patients often showed isolated abnormalities while clinically free from infection. In contrast, simultaneous depression of several parameters appeared to be associated with infection.*

## Introduction

The myelosuppressive and immunosuppressive effects of cancer chemotherapy are a major threat to the patient, infections being frequent, often severe, and sometimes fatal (Hui-Yen Chang et al., 1976). Attempts to monitor these effects have included studies of leucocyte number (Bodey et al., 1966), and of functions such as phagocytosis (Davies et al., 1976) or DNA synthetic activity (Han and Sokal, 1970). However, most studies have concerned one cell type or function in isolation. Because we consider it probable that host defence against infection is not simply related to numbers of any single cell or to any single function[1], we have attempted to obtain an overall view of the defence system during chemotherapy by measuring simultaneously both numbers and functions of the major types of leucocytes (neutrophils, monocytes, T and B lymphocytes). We have followed patients through courses of standard quadruple chemotherapy for advanced Hodgkin's disease with the aim of elucidating any patterns of abnormality and their relationship to therapy or infection.

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[1] Addison, I. E.: Host defence function: the problem of monitoring a system made up of interacting components. Manuscript in preparation

## Materials and Methods

*Patients.* 14 patients had Hodgkin's disease and were receiving chemotherapy. The age range was from 12–55 years. Some patients had relapsed with extensive disease after previous radiotherapy, while others were receiving drugs as primary treatment. Each patient was studied over several weeks, including at least one cycle of treatment, and most patients had received several cycles of treatment before the study period. Treatment was usually with standard MOPP or MVPP combinations (Nicholson et al., 1970; de Vita et al., 1970), each cycle lasting 14 days, but in some cycles chlorambucil 10 mg/day for 2 weeks was substituted for mustine (ClVPP). Drug dosage was adjusted according to the usual haematological criteria. Tests were made on the first day (day 0) just before treatment was given, and then on days 7 and 13. In some patients, tests were made between drug cycles as well. In all, 21 cycles were studied. Controls were normal individuals and patients with nonmalignant disease.

*Enumeration of Leucocytes in Whole Blood.* EDTA blood samples were used for conventional differential counts. Monocytes and T lymphocytes were counted by the nonspecific esterase method (Greaves et al., 1977) combined with staining for peroxidase, and B lymphocytes by staining for surface immunoglobulin (Pepys et al., 1976). Neutrophils were counted on blood films stained with May-Grünwald-Giemsa.

*Enumeration of Leucocoytes in Separated Blood.* Most studies of numbers of T and B lymphocytes in disease have used values derived from separated blood. Because this may involve a variable and differential loss of cells (Brown and Greaves, 1974), we made our counts on whole blood where possible. However, in the case of very lymphopenic patients the errors in counting small numbers outweighed the disadvantage of using separated cells, and we made our counts on a mononuclear preparation (Böyum, 1968), which allowed T cells to be estimated by sheep cell rosetting (Lay et al., 1971).

*Phagocyte Migration.* We used our modification (Addison and Babbage, 1976) of the membrane migration technique of Boyden (1962); this allowed the comparison of several samples on the same membrane.

*Neutrophil Bactericidal and Candidacidal Activity.* Pseudomonas aeruginosa and Candida albicans were selected as test organisms as they frequently cause infections in immunosuppressed patients. For the bacterial killing we used a modified version of the method of Grogan and Miller (1973) and for the yeast, the method of Lehrer and Cline (1969).

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**Table 1.** Normal range of leucocyte number and function

| | | |
|---|---|---|
| Polymorphs | Numbers | 3.1–6.65 × $10^9$/C |
| | Migration | 75–105 μ |
| | Candida killing | 15–25% of organisms |
| | Pseudomonas killing | 70–80% of organisms |
| Monocytes | Numbers | 0.25–1.05 × $10^9$/C |
| | Migration | 50–80 μ |
| | Candida ingestion | 60–80% of cells contained yeast: usually 3–5 per cell |
| T lymphocytes | Numbers | 1.0–3.6 × $10^9$/C |
| B lymphocytes | Numbers | 0.36–1.1 × $10^9$/C |

*Monocyte Phagocytic Activity.* Mononuclear cells, suspended in medium containing 0.1% BSA but no serum, were allowed to adhere to glass microscope slides for 30 min at 37° C. Nonattached cells were washed off and the adherent cells were covered with medium containing Candida albicans in the presence of either 10% autologous or control plasma. After a further 30 min at 37° C, free Candida was rinsed away with medium containing fluorescein diacetate and ethidium bromide, and the slide was then examined under the microscope using ultraviolet light with incident or dark ground illumination. Live cells are green (due to fluorescein liberated in the cytoplasm), while dead cells fluoresce red (due to the entry of ethidium bromide). Phagocytosed Candida show as dark spaces in the green cytoplasm. Candida outside the cells are invisible. The percentage of monocytes that had phagocytosed was counted.

*Expression of Results of Phagocyte Function Tests.* Because of the difficulty of reproducing absolute values in biological tests, even within the same laboratory and with carefully standardized techniques (Evans and Anderson, 1975), the results of function tests were expressed as a fraction of values obtained with a normal control included with each set of tests. Table 1 shows the range of control values in the nine tests used.

Initially, we have taken values less than 0.8 of the control to be abnormal, because when normals have been tested simultaneously all results have been within 20% of the extreme values.

## Results

### *Numbers of Circulating Cells during Chemotherapy*

The numbers of blood neutrophils and monocytes during chemotherapy are shown in Figure 1. The mean neutrophil count did not fall during treatment (because dosage reductions had often been made previously to avoid neutropenia). While the mean monocyte count did not fall significantly, the standard deviation increased greatly because some individuals developed very low counts by day 13. Figure 2 shows numbers of T and B cells during treatment. The number of T lymphocytes on day 0 was already lower than the controls, and during the cycle the mean count fell slightly; some patients, however, developed marked T-lymphopenia. A similar reduction in the number of B lymphocytes was seen in some patients, but again the mean counts fell only slightly.

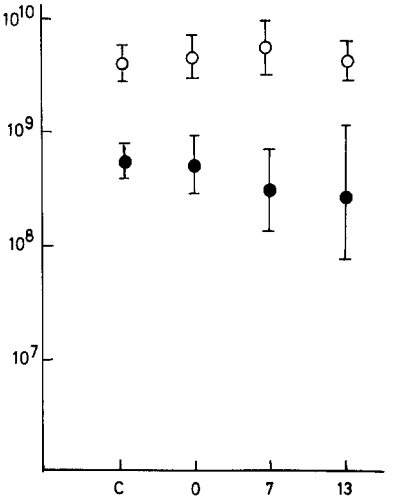


**Fig. 1.** Numbers of blood neutrophils (O) and monocytes (●) during chemotherapy. Values are geometric means and standard deviations of controls (C) and of patients on days 0, 7, and 13 of drug cycle

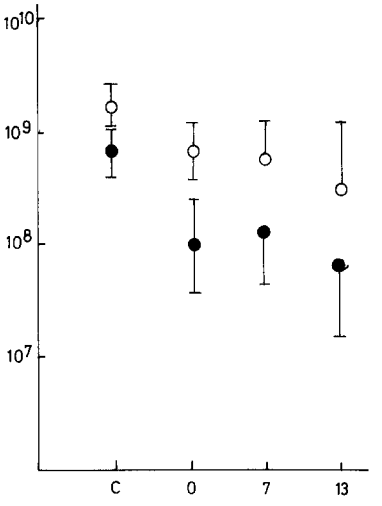


**Fig. 2.** Numbers of blood T lymphocytes (O) and B lymphocytes (●) during chemotherapy. Values are geometric means and standard deviations of controls (C) and of patients on days 0, 7, and 13 of drug cycle

### *Leucocyte Function Abnormalities*

The total number of abnormal tests on each of the 3 days in the cycle is shown in Table 2. Abnormal results were found frequently and throughout the cycle, but it was uncommon for more than one function test to be abnormal at a time. As most of the patients remained free of infection most of the time, abnormal results were often found in individuals who were free from infection. There was no general trend towards deterioration of function as the treatment cycle progressed.

When the five function tests were performed on leucocytes from 16 control patients, only one test was ab-

**Table 2.** Abnormal leucocyte function during MOPP therapy

| Day | 0 | 7 | 13 | Total abnormal |
|---|---|---|---|---|
| Polymorph | | | | |
| Migration | 10/18 | 5/19 | 5/11 | 20/48 (42%) |
| Candida phagocytosis | 6/15 | 5/17 | 8/9 | 19/41 (46%) |
| Pseudomonas killing | 8/14 | 9/14 | 5/11 | 22/39 (56%) |
| Monocyte | | | | |
| Migration | 5/17 | 5/15 | 3/12 | 13/44 (29%) |
| Candida phagocytosis | 8/18 | 5/16 | 4/11 | 17/45 (38%) |
| Total abnormal | 37/82 (45%) | 29/81 (35%) | 25/54 (46%) | – |

**Table 3.** Relationship of combined abnormalities in mononuclear cell numbers and phagocyte function to clinical infection

| | No. of tests | Infection |
|---|---|---|
| Numbers not decreased | | |
| Less than 75% of functions depressed | 13 | 0/13 |
| More than 75% of functions depressed | 30 | 0/30 |
| Numbers decreased | | |
| Less than 75% of functions depressed | 20 | 0/20 |
| More than 75% of functions depressed | 7 | 4/7 |

Criteria for depression of numbers and function given in text

normal on one occasion. The finding of abnormal tests in the Hodgkin's patients is therefore significant, even though there was no correlation with the treatment cycle.

### *Relationship of Abnormal Results to Infection*

During the study, four patients developed infections which required that the patient be admitted to hospital, treated with antibiotics, or temporarily spared chemotherapy. No single measure of numbers or function was

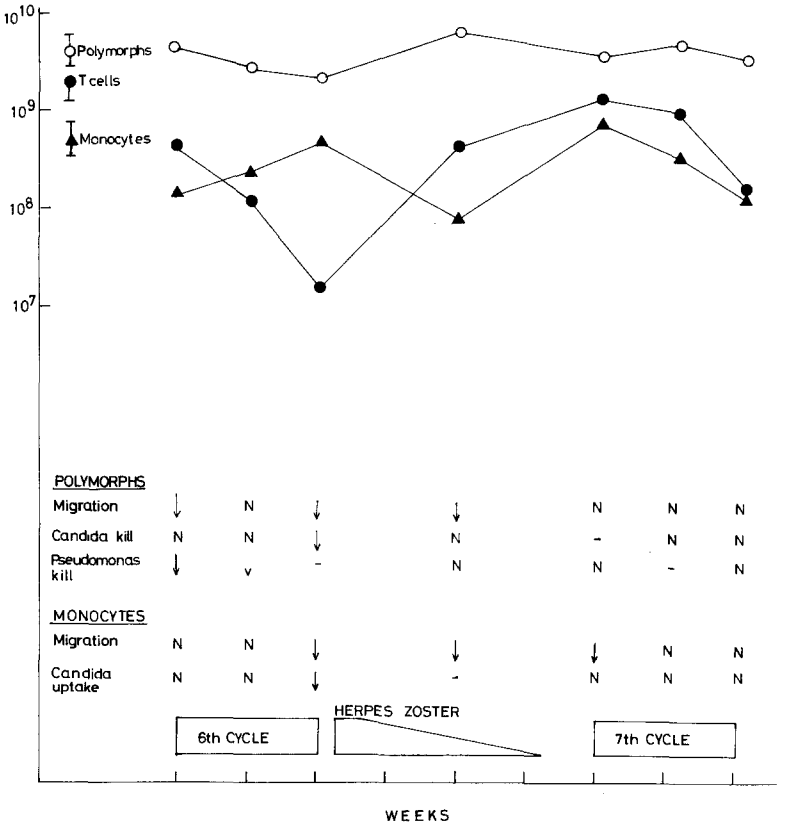


**Fig. 3.** Mrs. J. H. Leucocyte numbers and phagocyte function during chemotherapy. Vertical arrows indicate function depressed below 80% of normal; N indicates normal function. Normal values for leucocyte numbers are shown with standard deviations, next to vertical axis

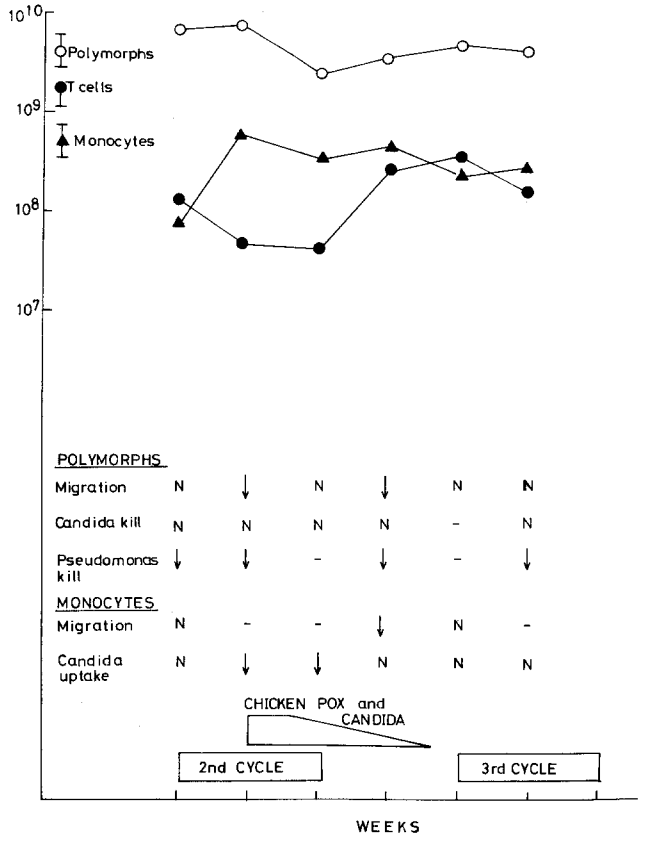


**Fig. 4.** S. M. Leucocyte numbers and phagocyte function during chemotherapy. Vertical arrows indicate function depressed below 80% of normal; N indicates normal function. Normal values for leucocyte numbers are shown with standard deviations, next to vertical axis

associated with these episodes; in particular, the neutrophil count was normal in each case. However, analysis of the results showed that in each of these patients in the test preceding the infection there was a reduction of one mononuclear cell count or more to less than 25% of the lower limit of the normal range and, simultaneously, 75% or more of the function tests were abnormal. The data for all of the tests are shown in Table 3 on the basis of these criteria (but excluding repeat tests on patients while they were infected). The Table shows that no infections occurred when three or more function tests were simultaneously abnormal without gross reduction in mononuclear cell numbers (30 occasions). Similarly, no infections occurred when there was reduction in numbers but abnormalities in less than 75% of the function tests (20 occasions). In contrast, infection occurred on four of the seven occasions when both numbers and function were depressed. It is of interest that in all these patients the polymorph count was within the normal range.

### *Illustrative Case Histories*

1. A 26-year-old woman with Hodgkin's Disease (pathological stage 4B) was started on quadruple therapy with ClVPP. After five cycles of treatment she still had occasional sweating at night and the ESR was high. The treatment-free interval was decreased from 4 weeks to 3 before the sixth cycle. The results of the tests during the sixth and seventh cycles are shown in Figure 3. T lymphocytes fell to 2% of the original value, while the neutrophil count remained normal and the monocyte count rose. At the end of the sixth cycle four function tests were abnormal. At this point she developed herpes zoster and generalised chicken pox. During the next 4 weeks the T cell count rose, two of the previously abnormal function tests became normal, and the rash faded. In the seventh cycle the T cell count fell to a lesser extent and there were some abnormal function tests, but no further infection occurred. The polymorph count was normal throughout.

2. An 11-year-old boy with stage 4B Hodgkin's disease was started on chemotherapy with MOPP. The tests done during the second and third cycle of treatment are shown in Figure 4. There was severe T- and B-lymphopenia, and in the middle of the second cycle there was no pseudomonas killing, and polymorph migration and monocyte phagocytosis of Candida were defective. At this point he developed chicken pox and oral Candida. The lymphocyte count recovered partially during the second treatment, and only one of the function tests (Pseudomonas killing) remained abnormal.

## Discussion

Infection has remained a major problem during intensive cytotoxic chemotherapy (Bodey et al., 1966) despite the practice of adjusting dosage on the basis of total leucocyte or neutrophil count. Moreover, infections frequently occur while neutrophil counts are normal. A variety of other tests have been used to see whether other aspects of host defence are damaged by treatment, including neutrophil phagocytic (Davies et al., 1976), bactericidal, and chemotactic activity and lymphocyte transformation (Han and Sokal, 1970). However, these studies have usually concerned only one cell type and only one function.

We suggest that the complex network of mechanisms which are important in defence against infection require to be studied by means of a comprehensive set of tests that measure the numbers and functional abilities of at least the major cell types involved. No single measurement on the blood is likely to correlate with infection unless the derangement is very great and prolonged (e.g., very low neutrophil counts), while simultaneous depression of numbers and functions of several cell types may cumulatively have a major effect on resistance to infection.

Previous studies of the effects of drugs on host defence have usually relied on single samples from patients on differing drug regimens. In this type of study no correlation of the tests with treatment or susceptibility to infection can be made. For this reason we selected a

group of patients with a tumour whose treatment is similar in most centres, and studied the patients serially throughout the treatment cycle.

The results have proved interesting in several ways. Firstly, there was considerable variation in the numbers of circulating T and B lymphocytes and monocytes before each cycle, representing the variable effects of disease and previous radiotherapy and chemotherapy. As treatment proceeded, the mean T cell and monocyte counts did not change greatly, but there was an increasing frequency of very low values, some T cell counts being reduced fifty-fold. The two patients with the lowest T cell counts had serious infections within a few days, one developing oral candidiasis and chicken pox and the other severe herpes zoster.

Secondly, isolated functional abnormalities were seen frequently in most patients while they were free of infection, and each function appeared to vary independently. These results support our suggestion that no single function can be relied on to predict the likelihood of infection. In tests on many other patients with nonmalignant diseases or in normal individuals we have seldom found abnormalities of function, and multiple abnormalities have not been seen.

Four patients developed severe or persistent infection. In each of these there was a combination of defects and depressions at the time when the infection developed, i.e., there was a depression of the count of at least one mononuclear cell type (to less than 25% of the lower limit of the normal range) combined with a simultaneous depression of phagocyte function. Grouping of the data from all the patients showed that a simultaneous depression of numbers and function of this degree occurred only seven times in 70 tests, and it is significant that all the serious infections of the series occurred in this subgroup. The infections occurred at a time when the neutrophil count was in the normal range. While the number of circulating neutrophils does not indicate the potential rate of production of these cells in response to infection, the neutrophil count is widely used as a guide to whether chemotherapy can be given safely. The studies reported here show that many other abnormalities in the host defence system develop during chemotherapy, which may increase susceptibility to infection, and suggest that our prediction of an association between multifactorial depression and the development of infection is worth further study.

Monitoring of host defence in this manner could provide information of value in analysing the harmful effects of chemotherapy and lead to less toxic schedules of administration of cytotoxic agents without loss of antitumour effect. The present panel of tests is an imperfect first attempt, and moreover is too arduous for routine work. Nevertheless, the results obtained justify the study of more, and different, patients in this way in an effort to produce a simpler but adequate defence screen.

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