Archivum Immunologiae et Therapiae Experimentalis, 2000, **48**, 195–200
PL ISSN 0004-069X

Circulating Immune Complexes in Advanced Hodgkin's Disease: Qualitative Analysis and Prognostic Significance

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Abstract. The level of circulating immune complexes (CIC) may be a reflection of the underlying malignancy and appears to be related to the stage of disease, tumor burden and prognosis. Prognostic factors at diagnosis, clinical response, survival and CIC were analyzed in 89 patients with Hodgkin's disease. All patients were newly diagnosed, in advanced stage and treated with MOPP regimen. The median follow-up was 41 months. CIC were estimated by the polyethylene glycol precipitation test. The median age was 40 years and 52% were under the age of 45. Nodular sclerosis and mixed cellularity were the most common histologies, 36 and 35% respectively. "B" symptoms were present in 65%, bulky disease in 29% and bone marrow involvement in 4% of the total. The erythrocyte sedimentation rate (ESR) was over 30 in 72% of patients and 27% had one or two extranodal localizations. Complete remission (CR) was obtained in 69 patients (77%). The only factor influencing the CR rate was the number of extranodal localizations ($p<0.05$). The ten-year relapse-free survival (RFS) and overall survival (OS) were 63 and 83%, respectively. RFS was adversely influenced by lymphocyte depletion histology ($p=0.009$) and by performance status over 1 ($p=0.003$). Elevated CIC levels were detected in 58% of the total. Patients with ESR over 30 had significantly higher values of CIC ($p<0.05$). Qualitative analysis of the CIC showed high levels of positivity to immunoglobulin G and M. C-reactive protein (CRP) was identified in 42% of all samples. CRP is an acute phase protein which shows conformational similarity to the immunoglobulin molecule. There were no significant correlations between levels of CIC and the other prognostic factors. Survival was not influenced by the CIC level.

Key words: Hodgkin's disease; immune complexes; prognostic factors.

Introduction

Hodgkin's disease (HD) patients frequently suffer from specific impairment of their immune system, shown by low T cell counts with a low CD4/CD8 ratio, an increase in spontaneous proliferative activity of lymphocytes, but a decrease in responses to mitogens and antigens and impaired delayed-type hypersensitivity reactions. It is also interesting to note that the lymphocytes surrounding Hodgkin/Reed-Sternberg (H/RS) cells in tissue sections do not exhibit a suppressor/cytotoxic phenotype, but rather show the phenotype of activated helper/inducer memory T cells ($CD4^+$/ /$CD45RO^+$). The fact that proven expression of Epstein-Barr-virus (EBV) proteins in H/RS cells, which may serve as a target for the cytotoxic T cells in an EBV-infected tumor cell, may reflect an underlying, possibly limited immune defect[6, 15].

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Classical prognostic factors for patients with HD are no longer major variables in predicting cure and survival, because current successful therapy has overcome these prognostic variables[18]. Systemic symptoms, erythrocyte sedimentation rate, albumin, lymphocytopenia, histologic subtype, multiple extranodal sites, the number of involved sites and specific disease sites such as pleura or liver have been identified as unfavorable prognostic factors in multiple published studies[5, 12]. Due to the natural history of HD and the differences in therapy, prognostic factors may be different in early versus advanced disease. In a recent report, STRAUSS et al.[19] defined serum lactate dehydrogenase, low hematocrit, age > 45 years, mediastinal mass ratio > 0.45, inguinal adenopathy and bone marrow involvement as poor prognostic indicators of freedom of progression in patients with advanced HD (stage IIB–IV). The same factors without marrow disease were adversely prognostic for survival.

A few studies analyzing immune deficiencies affecting the T cell response of HD patients pointed out the possible immunosuppressive role of circulating immune complexes (CIC) [2, 20, 21]. The role of immune complexes (IC) in HD has been reviewed under the aspects of detection of IC, clinical manifestation of IC deposition (renal and systemic manifestation), level of CIC and a possible pathophysiological role[9]. Despite the limitations in current methods of detecting CIC, the weight of evidence appears to favor the premise that elevated CIC levels are present in most patients with HD and appear to be related to the stage of disease and tumor burden. There is no clear data concerning a possible prognostic role of CIC in Hodgkin's disease[1, 4, 11].

The aim of this study is to determine the possible significance of pretreatment CIC level in predicting the outcome and survival of patients with advanced HD (stage IIB-IV) and also to analyze the composition of immune complexes in serum samples of patients with HD.

Materials and Methods

This study included 89 consecutive patients with previously untreated HD in advanced stage (IIB-IV) admitted to our hospital from 1985–1994. The main characteristics of the entire group are illustrated in Table 1.

The patients were divided into two groups: older and younger than 45 years. Forty-eight were older than 45 years, 54% were male. Most patients were in stage III of disease and only 4% with proven bone marrow infiltration. B symptoms were present in 65 and 29% had the bulky form of disease. Nodular sclerosis was the most common histology (36%). The erythrocyte sedimentation rate (ESR) over 30 mm/1 h was present in 72% and two or less extranodal localizations in 27% of patients.

Table 1. Patients characteristics

Patients characteristics	Number	%
Total	89	100
Age		
≤ 45 years	46	52
> 45 years	43	48
Male	48	54
Female	41	46
Stage		
II	29	33
III	42	47
IV	18	20
B symptoms	58	65
Histology:		
lymphocyte predominant	13	15
modular sclerosis	31	36
mixed cellularity	32	35
lymphocyte depletion	13	15
Bulky disease	26	29
Performance status		
0	46	52
1	34	38
2	9	10
ESR ≤ 30 mm/1 h	25	28
ESR > 30 mm/1 h	64	72
Bone marrow infiltration	4	4
Number of extranodal sites		
0	65	73
1	22	25
2	2	2
Level of circulating immune complexes		
normal	37	42
elevated	52	58

Median follow up for the entire group was 40.5 months (range 4–120 months).

The date of this analysis was November 1996.

Therapy administration and assessment of response. The initial anatomic extent of disease and response to therapy were assessed according to conventional clinical procedures. Patients were staged at diagnosis with disease history and clinical examination, pathological routine hematological and biochemical indices, chest X-ray, computerized tomography of chest and abdomen, unilateral bone marrow aspirate and biopsy and other tests as clinically indicated.

The originally described MOPP[7] chemotherapy was administered to the entire group, maximum 8 cycles.

Response to therapy was evaluated according to the WHO criteria. Factors influencing complete response (CR) rate were determined using the Pearson-χ^2 test. Overall survival (OS) and relapse-free survival (RFS) were calculated with the product-limit method and the significance of differences with the log-rank test. Univariate analysis was used for the analysis of covariance influencing RFS and OS.

Estimation and analysis of circulating immune complexes. Circulating immune complexes were measured using polyethylenglycol (PEG) precipitation test[17]. The method is based on a direct photometry measurement of turbidity formed in diluted human sera at 3.75% PEG concentration. PEG serum precipitation was done in 0.01 M borate buffer, pH 8.4, or 0.1 M phosphate buffer, pH 7.2, by addition of PEG 6000 solution to human sera to obtain the final 3.75% PEG concentration and a 1:30 serum dilution. The results were measured after 1 h incubation at room temperature and expressed in XXX value in comparison with the same serum concentration in buffer only. To obtain larger amounts of precipitates, 1–3 ml of the sera were treated similarly as in the standard routine test and after 60 min standing at room temperature the fine precipitate was collected by centrifugation at 2200 g for 120 min. The sediment was washed once with 3.75% PEG solution in borate buffer, recentrifuged and dissolved in a 6 M urea in glycine buffer of pH 2.8. This rapid and well reproducible test for detection of CIC in sera makes further determination of IC components easy and feasible. CIC were measured before initiation of therapy, after each cycle and, finally, after completing therapy.

The composition of soluble IC from fresh plasma samples was studied by combining several analytic immunological techniques. Anti-immunoglobulin antibodies were detected using RF latex and Waaler-Rose agglutination tests, C-reactive protein (CRP) was determinate also by the latex agglutination test. The originally described radial immunodiffusion test was used to determine and to semi-quantify the immunoglobulins A, G, M, D, the complement components C3, C4, C3a, fibrinogen, antitrombin-III, α_2-macroglobulin and α_1--antitripsin (Behringwerke®) [14].

Table 2. Results of therapy according to pretreatment variables

Patients characteristics	Number of complete remission	%
Total	69	77
Age		
≤ 45 years	35	74
> 45 years	34	81
Male	38	79
Female	31	76
Stage		
II	20	69
III	36	86
IV	13	72
B symptoms		
positive	45	78
negative	24	77
Histology:		
lymphocyte predominant	11	85
modular sclerosis	23	74
mixed cellularity	26	81
lymphocyte depletion	9	69
Bulky disease	19	73
Non bulky disease	50	79
Performance status		
0	37	80
1	26	76
2	6	67
ESR ≤ 30 mm/1 h	21	84
ESR > 30 mm/1 h	48	75
Bone marrow infiltration		
positive	3	75
negatives	66	78
Number of extranodal site		
0	52	80
1	17	77
2	0	0
Level of circulating immune complexes		
normal	31	84
elevated	38	73

Results

Efficacy and prognostic factors

Results of therapy according to pretreatment variables are illustrated in Table 2. Complete remission after 8 courses of therapy was obtained in 69 patients (77%). More than one extranodal localization of disease was the sole adverse factor for CR ($p<0.05$). Ten-year relapse-free and overall survival grates were 63 and 83%, respectively (Fig. 1, 2). RFS was adversely affected by lymphocyte depletion histology ($p=0.009$) and by performance status over 1 ($p=0.03$) (Fig. 3, 4). No one factor influenced overall survival.

Circulating immune complexes

Elevated level of CIC were detected in 52 patients (58% of the entire group). The complete remission rate was not influenced by CIC levels (Fig. 5). A difference in CR rate between groups with normal and elevated levels of CIC was not statistically significant. ESR > 30

Table 3. Qualitative analysis of circulating immune complexes in sera samples of Hodgkin's disease patients

Parameter	Method	Number of positive samples	% positive samples
C-reactive protein	Latex	13	42
Rheuma factor	Latex	0	
Immunoglobulin G	RID	23	74
Immunoglobulin M	RID	10	32
Immunoglobulin A	RID	0	
Immunoglobulin D	RID-LC	0	
C3 complement comp.	RID	3	10
C4 complement comp.	RID	5	16
C3a complement comp.	RID	0	
Fibrinogen	RID	0	
Plazminogen	RID	0	
α_2-macroglobulin	RID	0	
α_1-antitripsin	RID	0	

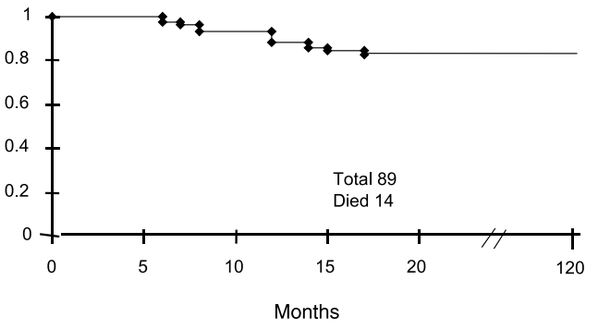

Fig. 1. Overall survival of Hodgkin's disease patients

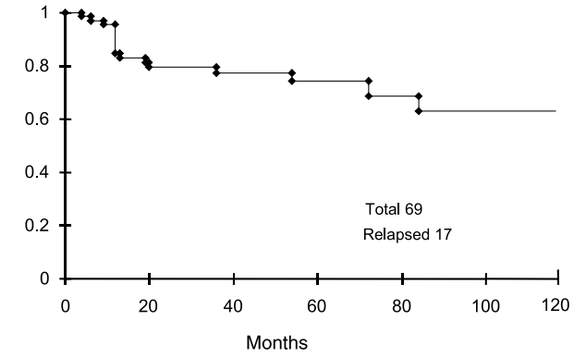

Fig. 2. Relapse free survival of Hodgkin's disease patients

mm/l h was the only adverse variable that positively correlated with elevated CIC level (p=0.027). Average values of CIC: before treatment, after each course and after chemotherapy, did not differ significantly (Fig. 6). RFS and OS were not influenced by CIC level.

Qualitative analysis of CIC

Results of sera sample testing are illustrated in

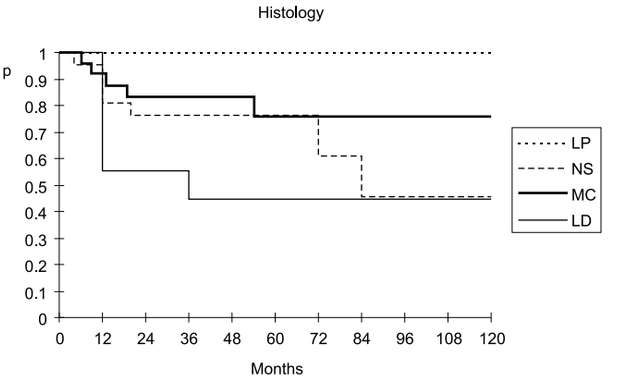

Fig. 3. Differences in relapse free survival according to pretreatment histology

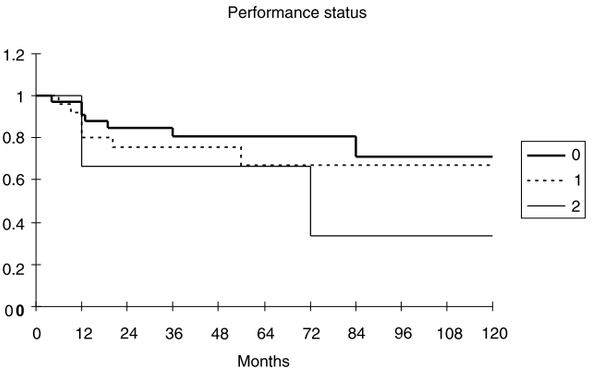

Fig. 4. Relapse free survival according to pretreatment performance status

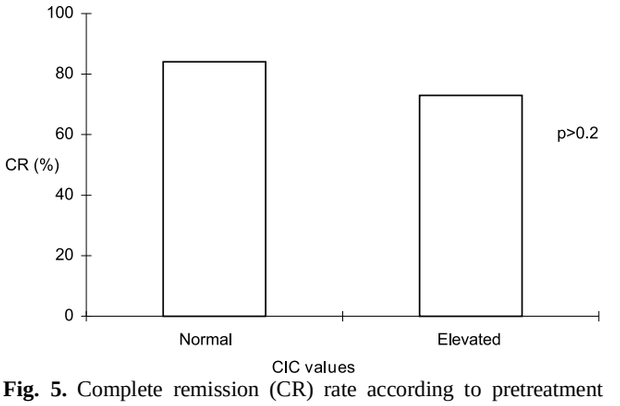

Fig. 5. Complete remission (CR) rate according to pretreatment level of circulating immune complexes (CIC)

Table 3. CIC were analyzed from 31 plasma samples of patients with HD. Nearly half of the samples (42%) were positive for CRP. Immunoglobulins G and M were positive in 74 and 32%, respectively. The complement components C3 and C4 were positive in 10 and 16% of samples, respectively. Rheuma factor was not detected in PEG precipitate using Waaler-Rose and latex RF agglutination tests.

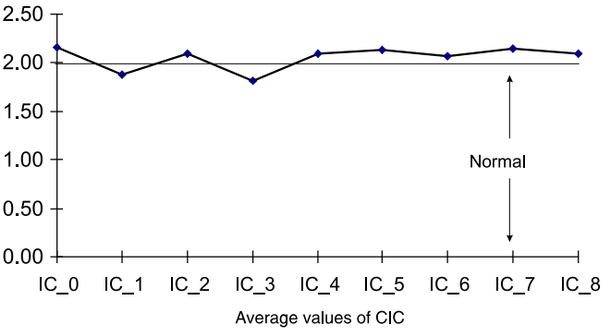

Fig. 6. Average values of circulating immune complexes (CIC) in each cycle of chemotherapy

Discussion

The results of this study indicate that in advanced Hodgkin's disease, MOPP chemotherapy produced a complete response in 77% of patients, with 10-year RFS and OS of 63 and 83%, respectively.

These results are comparable with those obtained by other investigators with the exception of RFS, which is in most studies over 70%. Despite the fact that most relapses occurred in the first 2 years, we noted a few late relapses of disease, the last, after 7 year of follow-up. Those patients with late relapses suffered from nodular sclerosis and lymphocyte depletion categories of histology.

The only factor influencing CR was the number of extranodal sites over 1, but there is no further proof of its influence on RFS and OS.

Relapse-free survival was adversely influenced by low performance status and lymphocyte depletion histology.

Overall survival from HD, as a crucial end point parameter, did not differ significantly among stratified groups according to pretreatment variables.

The previously noted difference in RFS and OS may suggest that a second therapy approach after MOPP failures can achieve good results in overall survival, but further confirmation we needed.

In order to better define prognostic subgroups of HD patients, we analyzed one of the "forgotten" parameters: circulating immune complexes, based on earlier works which showed elevated CIC levels in HD and also a correlation to tumor burden and stage of disease. Despite the limitations in current methods of detecting CIC, it is likely that the IC formed in cancer are of small size (possibly due to a large burden of tumor antigen) and that complexes in the sera of cancer patients generally are non-complement binding. The PEG precipitation method is a non-complement, physical, simple and reproducible test, useful for clinical routine testing. The PEG precipitation test is often believed to be non-specific, detecting not only circulating immune complexes, but other serum components, too. It was shown in earlier works that immunecomplexes were preferentially precipitated by PEG. These results were strengthened by the parallelism of results obtained by measurement of C1q bound to immune complexes and to the PEG test. Data presented by RIHA et al.[17] highlighted the usefulness and reliability of the PEG turbidity test for IC detection, based on the findings of immunoglobulin molecules, complement components and ultracentrifugated aggregates in PEG precipitates. On the other hand, several IC assays, based on interaction with complement (e.g. anti-C1qCIC, anti-C3dCIC, anti-C1qSPCIC) are limited to detection of complement fixing IC only. PEG precipitated material is also very suitable for further immunochemical analysis, which is not possible with other IC detection techniques.

In Hodgkin's disease there are conditions for generating other categories of protein complexes which can be detected in sera, such as the complex of CRP and β-lipoproteins, the complex of one category of high density lipoprotein (HDL_3) and amyloid protein SAA. We should not forget the often elevated concentrations of ferritin and α_2-macroglobulin in sera of patients with HD. We can presume that each of these categories may contributed to the well-known immune defect in HD, thus implying a need for their exact identification.

In our findings, elevated CIC level were detected in 58% of patients with advanced HD, but the difference between 2 groups, with normal and elevated CIC levels, was not statistically significant. We also noted that average CIC values did not change during and after chemotherapy. The somewhat better CR rate in the group with lower CIC levels was not statistically significant. CIC values had no influence on survival. In correlation tests between CIC levels and other classic prognostic factors, the only positive correlation we found was elevated CIC level and ESR over 30.

These results indicate that CIC determined by the PEG method should not be further considered as a prognostic factor, but only as a parameter of biologic tumor activity, probably in combination with methods which could detect other categories of immune complexes in sera[22]. This premise is supported by our qualitative analysis of CIC in HD. In half of the sera, CRP was detected. This finding very difficult to interpret. CRP is a macromolecule often elevated in the sera of patients with HD as a result of the increased production of interleukin 6.

Data from OSMOND et al.[13] have shown that CRP

shares homology in structure with immunoglobulin. On the other hand, CRP may serve as a complement activator. Our results have shown C3 and C4 complement positivity in 10 and l6% of sera samples, respectively, despite the fact that the PEG precipitation method is a non-complement binding test. These observations may, in some cases, explain high CRP positivity in PEG precipitates[8, 10].

The obtained results, therefore, suggest the possibility that detected complexes are not obviously immune complexes, but serum protein complexes of some other type.

In conclusion, clinicians are currently faced with a paradox of being able to detect levels of immune complexes in microgram quantities, but perhaps not always being readily able to interpret what these levels mean in a given clinical situation. A large body of literature describes the role of immune complexes in modifying the immune response, but direct proof of their immunoregulatory role in human malignancy is lacking[16]. It may be that immune complexes play on important role in the immune homeostasis of cancer. Manipulations that alter the balance of tumor antigen, antibody and immune complexes may have therapeutic consequences[3].

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Received in June 1999
Accepted in December 1999