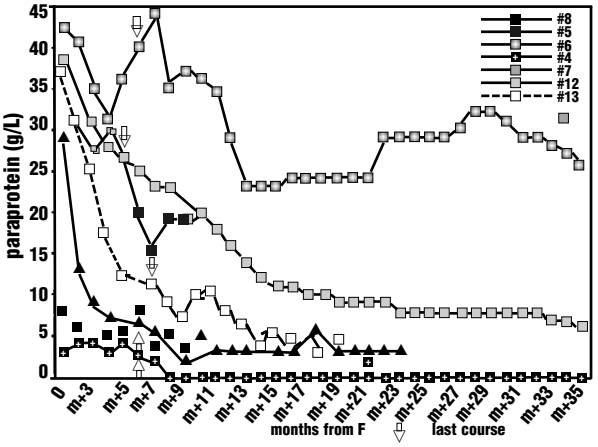

Figure 1. Levels of serum paraprotein during and after treatment with fludarabine or FC in the 7 patients with delayed response. Arrows indicate the last course of treatment.

od of at least 12 months from the end of therapy is required to determine chemosensitivity and maximum response to fludarabine or FC in LPL/WM. Response to therapy should be assessed at disease nadir to avoid missing delayed responses, as recommended by the Second International Workshop on WM.[6] However, neither response to fludarabine nor speed of response is a known predictor for progression-free survival and/or overall survival.[9]

Although treatment with nucleoside analogs in LPL/WM has been associated with a shorter time to response than treatment with chlorambucil, the occurrence of delayed responses, also described after fludarabine, cladribine and rituximab,[10] suggests that the biology of LPL/WM cells may be responsible for this phenomenon regardless of the type of chemotherapy given.

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Chronic Lymphoproliferative Disorders

Direct medical costs of mycosis fungoides in specialized Italian hospital departments

The objective was to analyze direct medical costs of managing mycosis fungoides (MF). We conducted a retrospective observational study in five Italian specialized departments. The 58 patients enrolled had a confirmed diagnosis of MF stage IIB or worse in 1999. The mean cost per patient was € 9,231.40.

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Mycosis fungoides (MF) is an epidermotropic cutaneous T-cell lymphoma (CTCL) causing proliferation of small or medium-sized neoplastic T lymphocytes with cerebriform nuclei.[1] The estimated annual incidence rate of MF is 0.3 per 100,000.[2-5] The treatment of CTCL varies according to the disease stage and the patient's age. Unfortunately, few patients achieve durable complete responses and cure of advanced disease is uncommon. To our knowledge, no article on the costs of CTCL has been published so far. This study is a first attempt to analyze resource utilization and direct medical costs of MF. A secondary aim of the study was to assess differences for clinically defined stages of disease.

We conducted a retrospective observational study in five departments specialized in the management of CTCL. Patients eligible for the study had a confirmed diagnosis of MF stage IIB or worse in 1999, and were still alive in March 2002. The time horizon of the study was two years (2000-2001). Data on patients' utilization of resources were extracted from medical charts and collected using a pre-designed questionnaire. Direct medical costs were estimated from the Italian National Health Service (INHS) perspective. Costs included outpatient and inpatient services delivered in the participating centers. Costs of incidental complications treated outside the participating centers

Table 1. Resource consumption per patient per year.

	MF stage IIB (n=30) mean (95% CI*)	MF stage >IIB (n=28) mean (95% CI*)	Total (n=58) mean (95% CI*)	P°
Laboratory tests	117.5 (81.0-178.5)	113.6 (69.9-183.2)	115.6 (85.6-156.3)	0.92
Diagnostic procedures	5.3 (3.8-6.8)	4.2 (2.8-6.1)	4.7 (3.7-5.9)	0.32
Specialist consultations	3.0 (2.1-4.4)	3.8 (2.6-5.3)	3.4 (2.6-4.3)	0.36
Day hospital (days)	22.3 (16.0-29.7)	14.3 (9.7-20.2)	18.5 (14.3-23.3)	0.08
Therapeutic procedures				
PUVA#	8.4 (4.4-15.0)	4.4 (1.9-9.7)	6.5 (3.8-10.4)	0.23
Radiation therapy	9.8 (5.9-15.6)	1.7 (0.4-3.9)	5.9 (3.7-9.6)	0.02
Phototherapy	0.7 (0.2-1.9)	1.2 (0.0-4.1)	0.9 (0.3-2.4)	0.58
Photopheresis	0.1 (0.0-0.8)	1.8 (0.9-3.3)	0.9 (0.5-1.8)	0.03
Full hospital admissions	0.5 (0.3-0.9)	1.1 (0.5-1.9)	0.8 (0.5-1.2)	0.17

**Bootstrap-t confidence interval (10,000 bootstrap replications); °bootstrap-t (10,000 bootstrap replications); #PUVA: psoralens + ultraviolet A radiation.*
The units of means are numbers, i.e. of tests, procedures, consultations, days, and admissions.

Table 2. Costs per patient per year (euros).

	MF stage IIB (n=30) mean (95% CI*)	MF stage >IIB (n=28) mean (95% CI*)	Total (n=58) mean (95% CI*)	p°
Laboratory tests	59.9 (34.2-129.6)	80.2 (30.4-212.1)	69.7 (41.7-133.4)	0.67
Diagnostic procedures	69.2 (34.2-140.2)	88.4 (39.1-199.9)	78.5 (46.8-137.9)	0.67
Drug therapy	274.5 (94.6-657.7)	408.8 (154.0-997.5)	339.3 (172.0-638.7)	0.58
Consultations	61.3 (43.7-90.2)	78.5 (54.2-109.2)	69.6 (54.1-89.7)	0.36
Day hospital days	6,062.5 (4,337.5-8,054.7)	3,890.6 (2,629.3-5,476.9)	5,014.0 (3,873.5-6,327.8)	0.08
Full hospital admissions	1,811.5 (1,034.3-3,120.1)	5,641.2 (2,873.4-9,832.4)	3,660.3 (2,216.4-5,910.7)	0.053
Total costs	8,338.9 (6,020.3-10,891.4)	10,187.7 (6,587.9-14,961.2)	9,231.4 (7,137.0-11,829.9)	0.45

**Bootstrap-t confidence interval (10,000 bootstrap replications); °bootstrap-t (10,000 bootstrap replications).*

were not included. Costs related to day hospital and full hospital admissions were priced using national tariffs from the Italian DRG-like system.[6] Consultations, diagnostic procedures and laboratory tests were priced by applying the INHS tariffs.[7] Dispensing prices were used for drugs.[8] The mean number of each resource consumed was multiplied by its tariff to calculate costs. Drugs costs were calculated by multiplying the number of days of therapy by the dispensing price. For the purpose of analysis we grouped patients in two diagnostic groups (IIB, >IIB) according to the stage at enrollment. Total costs were calculated for the two-year study period, but the results are presented as mean cost per patient per year. Since our sample was small and the distribution of costs was severely skewed, the total costs were compared using the bootstrap statistical method.[9,10] The participating centers recruited a total of 58 patients (range 9-22), 30 with stage IIB disease and 28 with more advanced MF. The mean age was 62.1 years, and the female to male ratio was 1:2.2. No patients were treatment-naive at enrollment. Eight patients received one treatment course during the two-year period and the other 50 more than one.

Table 1 shows the mean resource consumption per year. Patients with stage IIB MF received a significantly higher mean number of therapeutic procedures, especially first-line treatment (radiation therapy), whereas photopheresis and phototherapy were used more in patients with more advanced disease.

Table 2 shows the mean costs per year. Day hospital care was the major item of cost for patients with stage IIB disease, probably reflecting the greater use of therapeutic procedures in the early stage of disease. In contrast, costs of full hospital admissions were higher for patients in advanced stages, since they received more chemotherapy regimens or underwent transplantation. Broadly, the inpatient costs accounted for about 94% of total cost for both groups, confirming that outpatient costs play a minor role in this pathology. To further analyze costs in our sample, we compared the cost component by participating centers. The mean cost per patient varied significantly by center, ranging from 2,545.50 to 18,122.30 (p=0.0001). This suggests that medical management is still open to discretion at a local level. Potential limitations of this analysis should be borne in mind. First of all, participating centers were not randomly selected, and the study population consisted of patients attending referral centers, so they cannot be considered representative of all Italian patients. Nevertheless, the mean age at onset of disease and the sex ratio were consistent with those of the literature on this disease.[4] Secondly, resource consumption data were collected retrospectively. However, despite all the biases implicit in a retrospective study, the two-year follow up should strengthen the reliability of results. Thirdly, direct medical costs of incidental complications treated outside the participating centers were not included since such events, mainly infections, are usually treated in departments other than those specialized in MF. As complications are more likely in advanced stages, their exclusion would probably cause an underestimate of the cost difference between the two diagnostic groups compared. Lastly, unit costs were based on INHS tariffs rather than accurate estimates of real costs for each hospital. Being *lump sums* based on standard resource utilization, tariffs cannot capture the wide differences in diagnostic and medical procedures administered to MF patients in real practice.

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Malignant Lymphomas

Seminested polymerase chain reaction and heteroduplex analysis detects the monoclonality of *IgH* rearrangement in follicular lymphoma patients with high sensitivity

A new method, combining seminested polymerase chain reaction (PCR) with heteroduplex analysis, was utilized to detect follicular lymphoma (FL) cells in peripheral blood. The method, based on the detection of *IgH* rearrangements in DNA, detected the presence of monoclonal B cells in FL patients with a high frequency.

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The monoclonality of *IgH* gene rearrangements has been applied to distinguish malignant B cells from normal ones. The complementarity–determining region (CDR) in the variable region of the *IgH* gene is unique for each B cell clone and the CDR III is the clonal signature of an individual B cell.[1,2] The main difficulty of PCR-based clonality studies of B-cell malignancies is to discriminate between monoclonal and polyclonal products, especially when there is a high background of polyclonal B cells in the tumor sample. To discern between them more clearly, we utilized heteroduplex analysis in which PCR products are denaturated at high temperatures and subsequently renatured to induce homoduplex or heteroduplex formation. Genomic DNA was isolated from 24 patients (18 males, 6 females) with histologically verified FL in the Hematology and Medical Oncology Division of Hasan Sadikin Hospital, Bandung, Indonesia. Consensus PCR primers used for amplifying the *IgH* gene were: FR3A (framework 3A), FR1c (framework 1c) for the 3' end of the V region, and LJH (low JH), VLJH (very low JH) for the 3' end of the J region.[3,4] The first round of amplification was performed using an upstream consensus primer FR3A or FR1c and a downstream primer LJH that binds to all published JH gene segments. For reamplification, the lower strand primer (LJH) was replaced by a nested consensus JH primer (VLJH) and a small amount (1%) of the first PCR was used as the template.[5] The seminested PCR conditions were those described previously, with some modifications.[6] For heteroduplex analysis, PCR products were denaturated at 94°C for 10 min and subsequently cooled at 40°C to induce duplex formation. The hetero- and/or homoduplex (10 μL) were characterized by polyacrylamide gel electrophoresis (PAGE) with 10% non-reducing gel in 0.5 ×TBE buffer.[7] The gel was stained with a 1:1000 dilution of SYBR Gold dye in 0.5×TBE buffer for 30 min and visualized under ultraviolet light. One or two thin clear bands within the expected size (75-140 bp for FR3A and 330-350 bp for FR1c) were taken to signify positivity for clonality.[5,7] For sensitivity studies, peripheral blood mononuclear cells (PBMC) from normal subjects and DHL-4 cells (diffuse large B cell lymphoma) were mixed to make a ratio of one DHL-4 cell in 10^2, 10^3, 10^4, 10^5 and 10^6 PBMC and the total cell number was adjusted to 5×10^6. The DNA was extracted from the mixture using a Qiamp DNA Mini Kit (Qiagen).

The PCR products, loaded on 4% agarose gel and stained with ethidium bromide, are shown in Figure 1A. TK cells (diffuse large B cell lymphoma), used as the positive control (lane 2), and a FL patient (lane 4) showed a clear monoclonal band. On the other hand, normal PBMC as the negative control (lane 1) showed a broad band and FL patients (lane 3) had a very faint band. The seminested PCR alone gave a monoclonal band in 10 of 24 samples. However, it was very difficult to judge the presence of a clear monoclonal band in ethidium bromide-stained agarose gel. Therefore, heteroduplex analysis with the same samples shown in Figure 1A was performed by PAGE and visualized by SYBR Gold dye (Figure 1B). The monoclonal band was much easier to detect than with seminested PCR alone. A monoclonal band was seen in 19 of 24 patients (approximately 79%). Among the 24 FL patients, monoclonal *IgH* rearrangement was detected in 2 of 3 patients in stage I, both patients in stage II, 8 of 12 in stage III and all 7 in stage IV. In addition, there was no significant difference in clinical characteristics between patients with and without monoclonality. The method also identified monoclonality in NALM-6 (pre-B acute lymphoblastic lymphoma), RPMI 1788 (B acute lymphoblastic lymphoma) and HD-MY-Z (nodular sclerosis Hodgkin's lymphoma) cell lines. This combined method detected a monoclonal band in the DNA sample from the mixture containing 1 malig-