Eur J Cancer Clin Oncol, Vol. 22, No. 6, pp. 663–669, 1986.
Printed in Great Britain.

0277-5379/86$3.00+0.00
Pergamon Journals Ltd.

OKT4/OKT8 Ratio and Serum Beta 2- Microglobulin in Mycosis Fungoides and Chronic Benign Dermatitis

FILIPPO M. LARUSSA,* LUIGI M. LAROCCA,† LUIGI RUSCIANI,* LIVIO PAGANO,† GIUSEPPE LEONE,† ANTONIO VENIER* and FERDINANDO SERRI*

**Department of Dermatology and †Department of Hematology, Catholic University of the Sacred Heart, Rome, Italy*

Abstract—*Pre-treatment Serum Beta 2-microglobulin (S B2-m) and OKT4/OKT8 Ratio (T4/T8 R) were studied in 15 patients with Mycosis Fungoides (MF) and in 10 subjects with Chronic Superficial Benign Dermatitis (CSBD) in order to verify whether these parameters may lend support to an earlier differential diagnosis. S B2-m levels and T4/T8 R showed no significant difference in CSBD as compared to normal controls. MF patients displayed elevated S B2-m and T4/T8 R values in comparison to healthy controls and subjects suffering from CSBD ($P < 0.001$). After photochemotherapy (PUVA), markedly decreased S B2-m and T4/T8 R values were observed in all patients but two who proved to be unresponsive to PUVA treatment. On the basis of reported data, S B2-m and T4/T8 R can be regarded as an additional tool to discriminate CSBD and MF when clinical and histological features are not significantly diagnostic. Finally, these parameters seem to provide reliable information in monitoring response to treatment.*

INTRODUCTION

Mycosis Fungoides (MF) is widely recognized as the most common epidermotropic variant of Cutaneous T-Cell Lymphomas (CTCL). The term, introduced by Edelson [1], indicates a spectrum of disease entities which belong to the non-Hodgkin's Lymphomas (NHL). These malignancies are characterized by the neoplastic proliferation of a clone of helper lymphocytes which originate in, or predominantly involve the skin, and can be confined to the skin for many years. As a result, the earliest stage of the disease, premycotic or eczematous, according to Bazin's description [2], cannot be easily differentiated from those forms of Chronic Superficial Benign Dermatitis (CSBD) with the histological feature of a band-like mononuclear infiltrate [3, 4].

The therapeutic approach to MF includes photochemotherapy with 8-Methoxypsoralen and UVA (PUVA) and single/combined agent chemotherapy. The first modality has been used successfully in the treatment of early stage MF with a complete remission rate up to 62% [5]. In fact, a large percentage of MF patients run a chronic clinical course lasting 10–20 yr. A significant number of patients, however, have rapid clinical courses [5] with death from disseminated CTCL. In these cases, PUVA is insufficient, and systemic chemotherapy is required.

The distribution of peripheral blood T-cell subpopulations, determined by means of Monoclonal Antibody (MoAb) studies, can be a useful tool in the study, and even in the diagnosis, of CTCL, as previously reported [6]. To expand upon earlier studies, we utilized, in addition to OKT4, Beta 2-microglobulin, which has never before been used for the study of this group of diseases. B2-m is a low-weight protein (mol. wt 11,700) composed of 100 amino acids, the sequence of which is homologous to the constant region of IgG polypeptide chains [7]. B2-m is synthesized by most nucleated cells and constitutes the invariant light chain of serologically defined major histocompatibility antigens HLA-A, B, C [8]. Lymphoid cells and the reticulo-endothelial system, rich in HLA antigens, are probably the major sources of B2-m [8]. Elevated serum B2-m levels have been found in elderly patients and in those with renal impairment, autoimmune diseases and hematological malignancies, particularly lympho-proliferative disorders [9, 10].

In the present work, serum B2-m levels and T helper/suppressor ratio, as defined by MoAb, are investigated in MF patients in order to determine

Accepted 25 November 1985.
Correspondence and reprint requests to: F.M. Larussa, Department of Dermatology, Catholic University of the Sacred Heart Largo Gemelli, 8 00168 Rome, Italy
This work was partially financed by the Foundation for Research in Dermatology, Rome, Italy

whether these two parameters could provide additional reliable information of clinical value.

MATERIALS AND METHODS

Patients

Two groups of patients were investigated as well as a control group of healthy volunteers matched for age and sex. The first group consisted of 15 subjects (nine males, six females, mean age 48 ± 19 yr) affected by clinically and histologically confirmed MF. At the time of the study, none of these patients had received any type of therapy for at least 3 months. Staging was performed according to the modified Fuck's classification (12): Stage I: MF confined to the skin; Stage II: MF with dermatopathic lymphadenopathy; Stage III: MF with lymph node involvement; Stage IV: MF with visceral involvement. All 15 patients were found to be in Stage I of the disease. Assessment of S B2-m and T4/T8 ratio were performed before initiation of therapy and again after complete remission was achieved.

The second group was composed of 10 patients (7 males, 3 females, mean age 41 ± 12 yr) suffering from various forms of CSBD. Four were cases of large-plaque parapsoriasis, five were cases of chronic generalized eczema of unknown origin lasting for more than 4 months, and one was a case of lichen ruber planus. All 10, however, presented clinical and/or histological elements which were suggestive of MF. These patients had been treated only with topical steroids. At the time of the study, however, none of them had received treatment for at least 3 months. Serum B2-m and T4/T8 R were assessed at the beginning of the study.

Treatment of MF patients consisted of PUVA. Patients received constant doses of 8-Methoxypsoralen (0.6 mg/kg) 2 hr prior to UVA exposure. UVA was erogated by a PUVA Waldman 4001, which provides a continuous spectrum of 320–380 nm, with a peak irradiance of 4–6 mW/cm^2 at 365 nm, delivered uniformly over the entire cutaneous surface.

The initial UVA dose was between 1.5 and 3 J/cm^2 according to the patient's sunburn/suntan history. Exposure times were increased at each treatment by ⩾ 0.5 J/cm^2 depending on the presence of erythema. PUVA was usually given 4 times a week during the phase of lesion clearing. Once the MF lesions had cleared completely, patients were placed on a maintenance schedule. Therapy was then given once a week for 4 weeks and thereafter decreased to once every 2 weeks for 6 months. After 8.5 months, S B2-m and T4/T8 ratio were reassessed.

B2-microglobulin determination

Serum was obtained from all subjects by centrifugation at 2000 ***g*** for 10 min of venous blood kept for 2 hr at 37°C. Serum samples were stored at −40°C until assayed for B2-m. Serum levels of B2-m were determined by a solid phase radioimmunoassay (Phadebas, Pharmacia Diagnostics, Uppsala, Sweden).

Analysis of peripheral blood mononuclear cells

Peripheral Blood Mononuclear Cells (PBMC) were labelled by an indirect immunofluorescence technique: 100 μl of PBMC (10.10^6/ml in Hanks' medium 0.1% sodium azide) was mixed with 10 μl of antibody at different dilutions (1/50 for OKT3, 1/20 for OKT4, 1/50 for OKT8). After a 30-min incubation at 4°C, the cell suspension was washed and labelled with a fluorescein-conjugated F (ab') 2 goat anti-mouse IgG antibody diluted 1/10 (Cappel Laboratories, Cochranville, MD). One hundred microlitres of this antibody was added to the pellet and incubated for 30 min at 4°C. As a negative control, one sample was incubated without MoAb (non-specific ascites fluid). After two additional washings, the percentage of fluoresceinated cells was evaluated with an Orthoplan Leitz microscope. OKT3, OKT4, OKT8 MoAb's (Ortho Diagnostics, Raritan, NJ) were employed.

OKT3 reacts with 95% of peripheral T-cells [13]. OKT4 recognizes antigens expressed on approx. 55–57% of peripheral T cells, and OKT8, the determinants present on 20–30%. These two subpopulations include the inducer and the suppressor/cytotoxic subsets respectively [14].

Statistical methods

Single comparisons among the different groups were performed by Tukey's test. Unpaired test was used for B2-m and OKT4/OKT8 ratio (T4/T8 R) determinations in MF patients before and after therapy. Linear regression was assayed between S B2-m and T4/T8 R values of all Mycosis Fungoides patients before the initiation of PUVA therapy.

RESULTS

Therapy effectiveness

In 14 patients, cutaneous MF lesions disappeared completely after an average of 6.0 weeks (22.8 PUVA sessions; total UVA delivered 109 ± 22 J/cm^2). Clinical resolution of lesions was histologically confirmed in at least one site in each patient. One of these 14 patients suffered a relapse during maintenance therapy; restaging showed visceral involvement, and polychemotherapy was initiated using cyclophosamide, vincristine (Oncovin) and prednisone (COP). Reassessment of S B2-m and T4/T8 R was performed in this patient prior to the initiation of the cytostatic course.

In the fifteenth patient, PUVA therapy proved

ineffective as internal progression of the disease could be observed. The patient was promptly submitted to chemotherapy (COP), but he died after 4 months due to intercurrent infections.

Serum Beta 2 microglobulin

S B2-m levels in normal controls, CSBD and MF patients before therapy are reported in Table 1 and Fig. 1. No statistical difference was found between normal control values and those of the CSBD group. On the contrary, S B2-m levels were significantly higher ($P < 0.001$) in MF patients than in normal controls and in the CSBD patients. Serum B2-m values for the control and CSBD groups, as well as for the MF group before and after therapy, are reported in Table 1. Figure 2 shows S B2-m levels of the MF patients before and after PUVA therapy. All responsive MF patients showed a significant reduction of S B2-m values ($P < 0.001$) to within normal limits following therapy while the two patients who were unresponsive to PUVA showed increases in these values.

T4/T8 ratio

No statistical difference in the T4/T8 R was found between normal controls and CSBD patients, while T4/T8 R was found to be significantly increased when MF patients prior to therapy were compared to normal controls ($P < 0.001$) or to CSBD patients ($P < 0.001$) (Table 1 and Fig. 4). After therapy, responsive MF patients showed

*Table 1. Serum B2-m and T4/T8 R in all examined groups**

Populations	No.	S B2m	T4/T8 R
Normal controls	15	1.12 ± 0.40	1.23 ± 0.4
CSBD	10	0.89 ± 0.40	1.14 ± 0.30
MF before any therapy	15	2.62 ± 0.49	4.20 ± 1.46
MF after therapy	13	1.03 ± 0.34	2.19 ± 0.88

* Values are expressed as mean ± S.D.

T4/T8 R values significantly reduced as compared to those found before therapy ($P < 0.001$). Moreover, most of these values were within normal limits. T4/T8 R values in the two unresponsive patients, on the contrary, were found to be increased following therapy, as can be seen from Fig. 4.

DISCUSSION

Many investigators have attempted to establish well-defined, reliable markers for early diagnosis of Mycosis Fungoides. None of the presently available laboratory methods has proved to be completely satisfactory for this purpose. It is generally accepted that early MF lesions may be initially diagnosed as chronic non-specific dermatitis on the basis of light [15] and electron microscopic [16] features. DNA cytophotometry [17] and morphometric analysis [18] of lymphoid cells in skin infiltrates have proven useful in differentiating

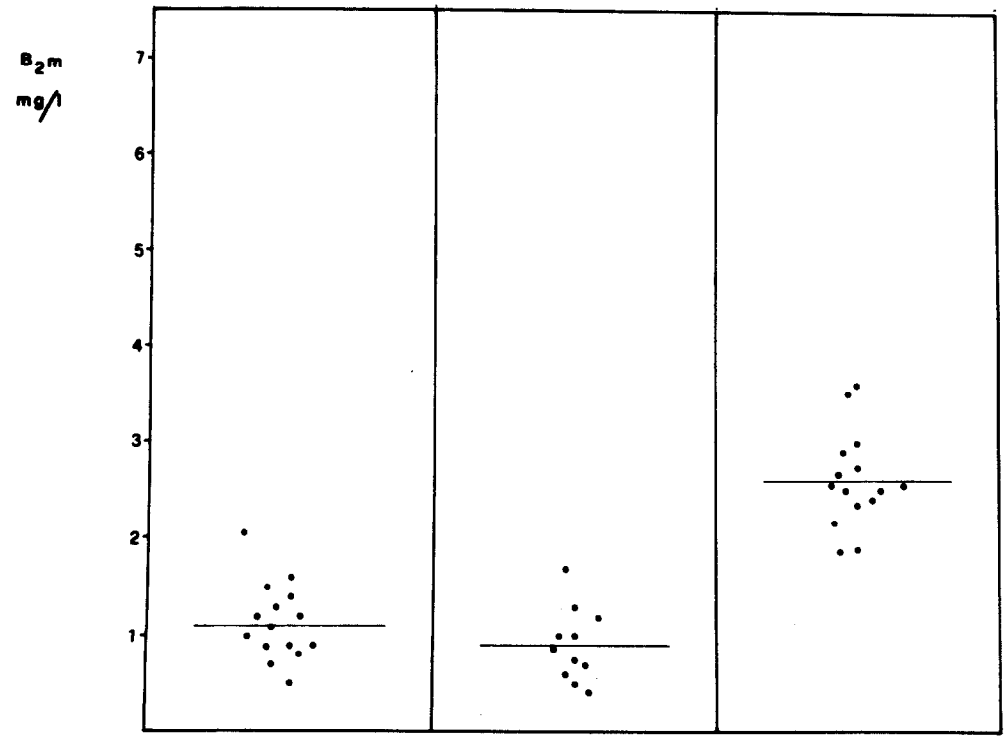

Fig. 1. Serum B2-m levels in (from left to right) normal controls, CSBD and MF patients before therapy.

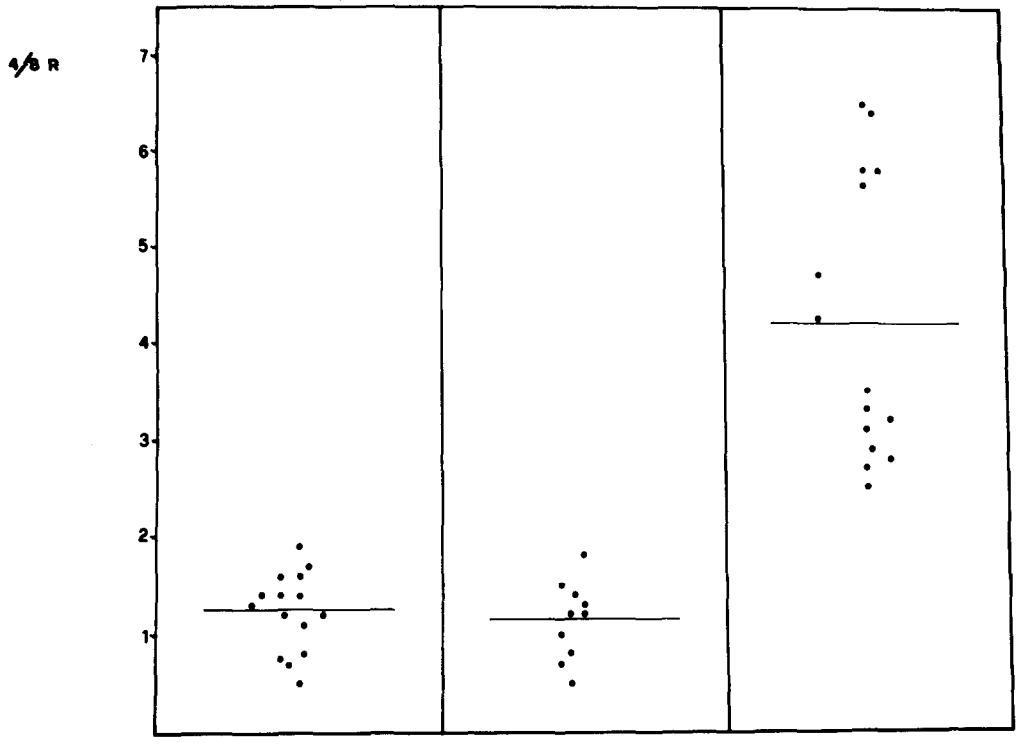

Fig. 2. T4/T8 R levels in (from left to right) normal controls, CSBD and MF patients before therapy.

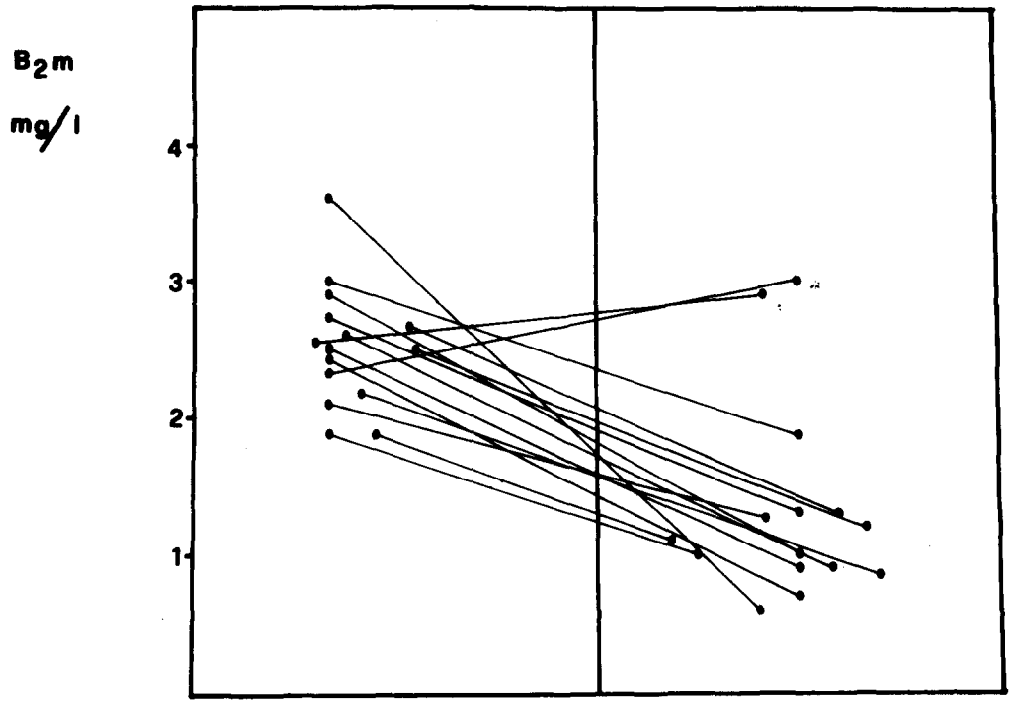

*Fig. 3. S B2-m levels in MF patients before and after PUVA therapy. * Unresponsive patients*

early MF from benign disorders [19]. These studies, however, are performed in only a few centers as they require skilled laboratory personnel and considerable outlay. More recently, immunohistochemistry methods using MoAb have been proposed to distinguish CTCL from benign conditions. Holden *et al.* [20] observed a selective loss of reactivity with monoclonal antisera OKT4 and OKT8 in a skin specimen of a CTCL patient. Unfortunately, this feature proved to be of limited

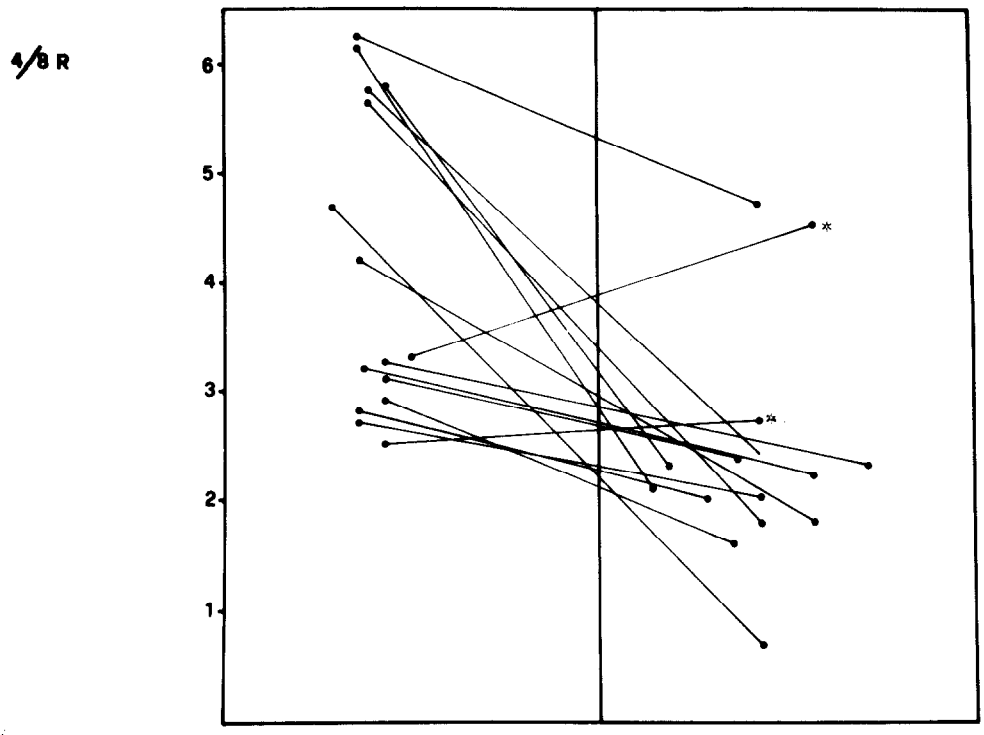

*Fig. 4. T4/T8 R values in MF patients before and after PUVA therapy. * Unresponsive patients.*

diagnostic value as it could be demonstrated only in advanced stages of the disease when clinical features were already indicative. Interesting data have also been reported by McMillan *et al.* [21, 22] who performed *in situ* studies of CTCL and large plaque parapsoriasis cutaneous infiltrates using immature and/or activation antigens of T-cell lineages T9 and T10. These results indicate that, while OKT10 positive staining cannot be considered a discriminant marker of CTCL, OKT9 reactivity may offer a possible alternative approach to the early diagnosis of this malignancy. It should be considered, however, that no details are furnished about the relationship between strength of T9 expression, degree of cellular proliferation and clinical staging. Furthermore, reported evidence [23] of OKT9 reactivity in lectin-stimulated T cells leads to the conclusion that this marker may also be found in various benign reactive disorders. Neither can the close functional relationship between lymphoid and Ia-like Ag positive-non-lymphoid cells in the early stages of CTCL, recently reported [24], be considered a diagnostic marker of the disease.

T lymphocyte subsets in peripheral blood have also been studied in CTCL patients. Recent observations by Haines *et al.* [26] support the suggestion that phenotypic changes producing surface antigen expression in circulating T cells depend upon the cutaneous microenvironment. Nevertheless, it must be stressed that studies of blood and tissue T cell subsets in CTCL patients will not be comparable until the role of the skin in extrathymic T cell differentiation has been completely defined.

Previous literature data on peripheral blood T lymphocyte distribution in CTCL patients has emphasized that an abnormal T4/T8 R is only a non-specific supplementary marker consistent with late stage CTCL [27]. On the other hand, T cell imbalance, similar to that we could demonstrate, has been described by Laroche and Boch in different stages of MF [28].

Our findings on peripheral blood lymphocyte distribution are not comparable with previous studies [19, 20] in which early stage patients are not included and MF patients on systemic therapy are, instead, considered. All of the MF patients in our study were in the early stages of their disease who had been off systemic therapy for at least 3 months. T4/T8 R values in these patients were found to be significantly higher than those of the control group or of the CSBD group. In addition, PUVA therapy produced a significant reduction in these values in all but two subjects who proved to be unresponsive.

The recent availability of BE1 and BE2 MoAb reactive with the tumor-associated antigens on the surface of the CTCL lymphocytes [30] allows an unambiguous diagnosis of CTCL in that they cannot be detected in either the cutaneous infiltrate [31] or peripheral blood of patients with benign dermatoses. Further information on the specificity of these two new MoAbs will be necessary [32].

Increased pre-treatment S B2-m levels in NHL patients have been reported to be associated with

advanced stages of the disease in several studies [33–35]. Numerous determinations of S B2-m levels have been made by these same authors. Amlot and Adinolfi [33] found a reduction in S B2-m levels after therapy even in the presence of recurrent or persistent disease, whereas Child [34] and Hagberg [35] found good correlation between the clinical course and S B2-m levels.

In the present study, elevated levels of S B2-m were found in all pre-treatment MF patients, but not in CSBD patients. In patients who achieved a completed remission, these values returned to normal. In the two patients who proved to be unresponsive to PUVA therapy, on the contrary, elevated S B2-m levels persisted. These two patients died after a few months, in fact, in spite of polychemotherapy.

The data reported here strongly suggest that the parameters under consideration may offer an important contribution to the differential diagnosis betwen CSBD and MF by providing an additional tool for the early identification of potentially malignant behaviour of the disease. Furthermore, sequential determinations of T cell subset distribution and serum beta 2-microglobulin could be useful in monitoring the patient's response to therapy.

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