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# Rapid immunochromatographic tests –an aid to the diagnosis of Dengue fever. A study conducted at Goa Medical College

KEYWORDS: Dengue, early diagnosis,NS1antigen, clinical profile


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**ABSTRACT** *Background: Dengue an arboviral disease found in the tropical and subtropical regions of the world is on the rise in our country. Early diagnosis is important to reduce morbidity and mortality.*

*Aims and objectives: 1) To study the prevalence of dengue based on the rapid screening tests like NS1 antigen, IgM, IgG antibodies and correlate with MAC-IgM ELISA.*

*2) To study the clinical pattern of dengue fever in patients attending a tertiary health care centre In Goa*

*Methods: 126 patients who presented with dengue like illness to Goa Medical College and were NS 1 antigen/ IgM/ IgG / MAC IgM positive were included in the study.*

*It was a retrospective study conducted between July 2014- June 2015. All the relevant data was collected from the case papers obtained from the Medical Records Department.*

*Results: Of the 126 patients included in the study, 101 patients (80.2%) were NS 1 antigen positive. 19 patients were rapid IgM positive (15%) and 6 patients were IgG(4.8%). Mac-IgMELISA was positive in 73 (57.9% ) patients, and 63(50%) patients were both NS 1 and IgM positive.*

*The rainy season July-September (34.1%) and post rainy season October to December (34.9%) had the maximum number of admissions. Patients in the age group 20-29 years (38.9%) were maximally affected. Males (74.5%) were affected more than females (25.5%) Fever, arthralgia rash myalgia petechiae and bleeding manifestation were the main clinical features seen in patients .Ascites and Pleural effusions were seen in 3 (2.4%) patients. Hypotension and acute renal failure occurred in 4 (3.2%) patients. Five (4%) patients developed ARDS. 3 patients died .Mortality being 2.4 %.*

*Leukopenia (83.3% ) thrombocytopenia (75.4%) and raised liver enzymes (96 % )were seen in most patients. 7 (5.6%) patients had PCV more than 50.Raised renal parameters were seen in 4 (3.2%) patients.*

*Conclusion Rapid ICT tests specially NS1 Antigen are useful markers in the early suspicion of dengue fever in patients with typical clinical and laboratory features specially in resource poor areas .Early diagnosis along with proper monitoring and prompt management helps to reduce the morbidity and mortality associated with the disease.*

**Introduction**

Dengue fever is caused by an arbovirus belonging to the Flaviviridiae family.[1] It is transmitted by the Aedes mosquito namely AedesaegyptiAedesAbolipticus and Aedes Polynesiensis.[2] The mosquito is a day biting mosquito which breeds in stagnant and stored water.

The disease has spread to most of the tropical and subtropical regions of the world. The incidence has increased 30 fold over the last 50 years mainly due to unexplained urbanisation with poor sanitation facilities[3]. In the developing countries Dengue still remains underreported due to poor diagnostic facilities.

In India Dengue was first reported from Vellore in Tamil Nadu in 1956 while Dengue Haemorrhagic fever first occurred in Calcutta in 1963 wherein 30% patients showed haemorrhagic manifestations.

There are four serotypes of Dengue virus DENV 1, DENV 2, DENV3, DENV 4. Following an infection lifelong immunity develops against the respective serotype. Immunity against other serotypes is limited and transient.[3]

The clinical spectrum of dengue infection can vary from an asymptomatic infection to an undifferentiated fever (viral syndrome), Classical Dengue fever with or without haemorrhage, Dengue haemorrhagic fever, dengue shock syndrome and expanded dengue syndrome (isolated organopathy / unusual manifestations .

Classic Dengue fever presents with high grade fever, rash, severe headache, body ache joint and bone pains ( break bone disease) .Bleeding manifestations may occur. Leukopenia and thrombocytopenia are commonly observed.

Dengue haemorrhagic fever occurs in patients who have been infected previously with a different serotype Patients present with high fever, haemorrhagic manifestations and circulatory disturbances Thrombocytopenia with a rising haematocrit is a constant finding in these patients. Abnormal haemostasis and plasma leakage in pleural and abdominal cavities are the main pathophysiological hallmarks of Dengue haemorrhagic fever. . Activation of the immune system leads to exaggerated cytokine response which results in increased vascular permeability . Complement activation specially by viral products like NS1 also lead to increase vascular permeability. Due to vascular leak patients may experience shock and organ impairment (Dengue Shock Syndrome.)[5]. With proper treatment if the shock is overcome patients may recover within 2-3 days. Mortality is high in this group if patients develop prolonged shock

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and multiorgan failure.The mortality from complications of dengue is as high as 20% but drops to less than 1% if recognised early and properly managed[1].

Unusual manifestations with severe organ involvement like liver, kidney, heart, brain have been reported in patients with dengue fever. These may be a consequence of prolonged shock or coinfection or comorbidities.

Diagnosis of Dengue can be done by isolation of the virus in cell cultures, detection of viral RNA by reverse transcriptase, detection of NS1 antigen and detection of IgM and IgG antibodies which are specific for virus envelope protein E.

Virus isolation using cell cultures and viral RNA detection are useful in acute stages of Dengue ie 0-5 days post onset however these tests are expensive and require expertise and appropriate facilities which are not easily available .NS1 Ag detection is a simplified method useful during the acute stage. NS1Ag, a highly conserved glycoprotein which is produced in both membrane associated and secretory forms by the virus is abundant in the serum of patients during early stages. High concentration can be detected in patients with primary or secondary dengue from day one up to 9 days post onset of symptoms using Elisa techniques and rapid immunochromatographic assays.

Antibody assays are based on the development of IgM and IgG anti dengue antibodies .In primary infection IgM is detected by the 5th day of illness and is detectable for up to 90 days .IgG becomes detected by day 10 -15 of illness and rises slowly . In secondary dengue infection IgM levels are low .High levels of IgG are detectable even in the acute phase and they rise dramatically over the next two weeks.

IgM capture Enzyme linked immunosorbant assay MAC – ELISA format is most commonly employed in diagnostic laboratories. The assay is based on capturing IgM antibodies on a microtitre plate using anti human IgM antibody followed by the addition of dengue virus specific antigen (DENV 1-4 ) .It has a sensitivity and specificity of 90% and 98% respectively when used 5 or more days after the onset of illness. Elisa based IgM is also an invaluable tool for surveillance of dengue infection. According to National Vector Borne Disease Control Programme (NVBDCP) MAC–ELISA should be considered as the diagnostic test for dengue infection.

**Materials and methods**

The study was a record based descriptive study conducted in patients admitted to the Medicine Department between July 2014 and June 2015 at Goa Medical College. 126 patients were included in the study. These patients presented to the hospital with clinical features suggestive of dengue and were NS1 Ag /IgM /IgG positive by the rapid Immunochromatographic tests.They were subjected to MAC Elisa test after admission .The details of clinical features, laboratory findings and complications were obtained from the case papers. Patients who tested positive for malaria, viral hepatitis, leptospirosis or any haematological disorder were excluded from the study.

Statistical analysis was done using hand calculator and Microsoft excel. Percentages and averages were calculated.

**RESULTS**

**Table 1 Age Sex distribution**

| Age | male | female | total | percentage |
|---|---|---|---|---|
| 12-19 | 10 | 2 | 12 | 9.5 |
| 20-29 | 40 | 9 | 49 | 38.9 |
| 30-39 | 25 | 6 | 31 | 24.6 |
| 40-49 | 9 | 5 | 14 | 11.1 |
| 50-59 | 8 | 5 | 13 | 10.3 |
| 60-69 | 1 | 5 | 6 | 4.8 |
| 70-79 | 1 | - | 1 | 0.8 |
| total | 94 (74.5% | 32 (25.5%) | | |

**Table 2 Seasonal variation**

| Seasonal variation | No of cases | Percentage |
|---|---|---|
| July to September | 43 | 34.1 |
| October to December | 44 | 34.9 |
| Jan to march | 12 | 9.5 |
| April to June | 27 | 21.4 |

**Table 3 Duration of fever**

| Duration of fever | no | Percentage |
|---|---|---|
| 1-5 days | 103 | 81.7% |
| 6-10 days | 21 | 16.7% |
| >10 days | 2 | 1.6% |

**Table 4 Clinical Presentation**

| symptoms | No of patients | Percentage |
|---|---|---|
| Fever | 126 | 100 |
| Myalgia | 81 | 64.3 |
| Rash | 58 | 46 |
| Vomiting | 36 | 28.6 |
| Arthralgia | 23 | 18.3 |
| Headache | 15 | 11.9 |
| Diarrhea | 9 | 7.1 |
| Abdominal pain | 6 | 4.8 |
| Petechiae | 38 | 30.2 |
| Bleeding | 18 | 14.3 |
| Pleural effusion / ascites | 3 | 2.4 |
| Renal failure | 4 | 3.2 |
| Hypotension | 4 | 3.2 |
| ARDS | 5 | 4 |

**Table 5 Serological markers**

| Marker | No of patients | Percentage |
|---|---|---|
| NS1 | 101 | 80.2 |
| IgM RAPID | 19 | 15 |
| IgG | 6 | 4.8 |
| NS1 and IgM rapid | 27 | 21.4 |
| MAC ELISA | 73 | 58 |

**Table 6 Investigations**

| investigation | No of patients | Percentage | |
|---|---|---|---|
| Hb<10gms | 6 | 4.8 | |
| Hb>16gms | 3 | 2.4 | |
| PCV,30 | 6 | 4 | |
| >30 | 7 | 5.6 | |
| WBC COUNTS | | | |
| <4000 | 105 | 83.3 | |
| LFT | | | |
| SGOT>40 | 121 | 96 | |
| SGPT>40 | 108 | 85.7 | |
| Serum bilirubin1-2mg | 5 | 4 | |
| Platelet count | | | |
| 50,000-100000 | 39 | 31 | |
| <50000 | 56 | 44.5 | |

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|---|---|---|---|
| Renal parameters | | | |
| Blood urea >40 | 4 | 3.2 | |
| Se creatinine>1.2 | 4 | 3.2 | |

126 patients were included in the study. Age sex distribution is seen in table 1. NS1 Ag was positive in 101 patients (80.2 % ).IgM rapid test was positive in 19 (15 % ) patients and IgG in 6 patients ( 4.8 % ).NS1 Ag plus IgM rapid were positive in 27 (21.4 % ) MAC Elisa was positive in 73 patients (58 % ). Of the 126 patients, 43 (34.15% ) patients presented during the rainy season from July to September and 44 (34.9% ) presented between October to December. There was a drop in number of cases with only 12 (9.5% ) from Jan to March.

Maximum number of cases ( 38.9% ) were in the age group of 20 to 29 years .There were only 6 (4.8 % ) patients between 60 to 69 years and only one patient ( .8% ) above 70 years.

94 patients (74 .5 % ) were males and only 32 (25.5 % ) were females. Most patients i.e. 103 patients (81.7 %) presented within 5 days of onset of fever.21 patients (16.7 % ) between 6 to 10 days and 2 ( 1.6 % ) patients with more than 10 days fever.

Fever ( 100 % ) was the commonest manifestation followed by myalgia in 81 (64.3 % ) patients , maculopapular rash (46 % ) , vomiting ( 28.6 % ) , arthralgia (18.3 % ) , headache (11.9 % ), diarrhoea (7.1 % ), abdominal pain (6 % ) .Petechiael haemorrhages were seen in 38 patients (30.2% ) while bleeding manifestations in the form of subconjunctival bleeds, gum bleeds, epistaxis ,upper GI bleeds, genitourinary bleeds occurred in 18 patients ( 14.3 % ).Ascites and pleural effusion were found in 3 patients (2.4 % ).Hypotension and Renal failure developed in 3 patients (2.4 % ). Five (4 %) patients developed ARDS ( adult respiratory distress syndrome ).Mortality was 2.4 % (3 patients ).

Laboratory investigations showed a Haemoglobin of less than 10 in 6 pts ( 4.8 % ) and more than 16 in 3 patients (2.4% ).The PCV was less than 30 in 6 patients (4.8 % ) and more than 50 in 7 patients (5.6 % ) .Majority of the patients (83.3 % ) had a total leucocyte count of less than 4000 cells / cmm..About 95 patients (75.4 % ) had a platelet count of less than 1 lakh of which 39 patients (31 % ) had a platelet count between 50,000 to 1 lakh and 56 patients (11.1 % ) less than 50,000. Raised liver enzymes were seen in patients with Dengue with 121 patients (96 % ) showing a rise in SGOT and 108 patients (85.7 % ) a rise in SGPT .Only 5 patients (4 % ) had a rise in bilirubin between 1-3 mg %.Blood Urea and Serum creatinine were high in 4 patients (3.2 % ).

**Discussion**

Dengue is emerging as one of the most abundant vector-borne disease globally. Although, most infections are asymptomatic, or a brief systemic viral illness, a small proportion of patients develop potentially fatal complications. These severe manifestations, including plasma leakage, coagulopathy sometimes accompanied by bleeding, and organ impairment, occur relatively late in the disease course, presenting a window of opportunity to identify the group of patients likely to progress to these complications. However, as yet, differentiating this group from the thousands of milder cases seen each day during outbreaks remains challenging, and simple and inexpensive diagnostic strategies are urgently needed in order to improve case management and to facilitate appropriate use of limited resources. In conjunction with clinical and epidemiological surveillance, the early detection of dengue circulation or an increase in dengue activity helps health authorities get useful information on time, location, virus serotype and disease severity. The use of good dengue diagnostic tools is critical for laboratory confirmation of DHF/DSS, including the number of case fatalities, determining which strains are involved, and to derive estimates of total incidence following epidemics[1].

Dengue diagnosis is of major importance for research into host, virus and vector characteristics, for determining the epidemiological conditions influencing the pathogenesis of the disease, and for vaccine evaluation [11]

In this study 126 patients who had symptoms of an acute febrile illness with clinical symptoms suggestive of Dengue infection were included in the study. Common manifestations of early dengue are fever joint pains and rash[12].The initial marker to confirm the diagnosis of Dengue used here was the NS1 antigen test. This was found to be positive in 80% of the patients at time of admission.

In a study by Datta et al New Delhi NS1 Ag positivity was 71.42% in acute phase sera[6] .It substantiates that in comparison to MAC-ELISA, NS1 Ag assay is an effective tool for diagnosis of DV infection, especially within the first four days of illness. Early detection of DHF by NS1 assay can help in early confirmation and management of this vulnerable group. NS1 Ag assay, if used in combination with MAC-ELISA on a single serum sample of a suspected case, has the ability to improve the diagnostic algorithm contributing significantly to the clinical treatment and control of dengue viral infections[6] Subhamoy Pal et all studied the efficacy of NSI RDT in South America and found the sensitivity of the RDTs ranged from 71.9%–79.1% while the sensitivity of the ELISAs varied between 85.6%–95.9%, using virus isolation as the reference method[13]

Detection of the DV non-structural protein 1 (NS1) has emerged as an alternative biomarker to both serologic and molecular based techniques for diagnosis of acute DV infection. NS1 antigenemia is detectable within 24 hours and up to 9 days following symptom onset. This overlaps with the DV viremic phase and NS1 is often detectable prior to IgM seroconversion[14]. In the present study it was seen that the IgM test on same day was positive in only 15% of the patients and IgG was positive in 4.8% of the patients correlating with the above statement.

In primary infections, immunoglobulin M (IgM) is detected 5 or more days after the onset of illness in the majority of infected individuals and immunoglobulin G (IgG) is detected from 10–15 days[14] Serological tests are more commonly used to diagnose Dengue infections because of their ease of use compared to techniques such as cell culture or RNA detection In secondary infections, IgM appears earlier or in the same time frame but are usually at lower titres than in primary infection. IgG is present from the previous infection and the titre increases rapidly[14].

In a study by Elizabeth et al NS1 tests were generally more sensitive in specimens from the acute phase of dengue and in primary DENV infection, whereas IgM anti-DENV tests were less sensitive in secondary DENV infections. The reproducibility of the NS1 RDTs ranged from 92-99% and the IgM anti-DENV RDTs from 88–94%[15] .Hence concurrent

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evaluation for the NS1 antigen alongside testing for IgM and IgGclass antibodies to DV provides optimal diagnostic potential for both early and late dengue disease[6]

In a study by Kassim FM et all however 32.2% were found positive for dengue NS1 antigen, 38.5% were PCR positive, 40.9% were IgM positive and 36.1% were IgG positive for dengue virus. The results reveal the detection rate of dengue virus infection was similar for PCR and dengue antibody (65.9%) and for NS1 antigen and dengue antibody (62.0%) combinations[16] Therefore, the dengue NS1 antigen test can be used to complement the current antibody test used in peripheral laboratories .Murtuzaetal found NS1 antigen reactive patients found more in number when compared to seropositiveIgM and IgG antibody patients[2]. A similar study was conducted by Anugha G. Kinikar et.al shows alike results[31].

Thus, the combination of the NS1 antigen and antibody tests could increase the diagnostic efficiency for early diagnosis of dengue infection. In the present study Mac Elisa was positive in 73 patients which was 58% as compared to NS1 which was positive in 80.2% . Since Mac Elisa may become positive on the5th day , and we may have lost patients to early discharge who could not be followed up for a repeat Elisa test . CDC describes Mac Elisa as having a specificity of 98% and sensitivity of 90.3%[17].The Dengue IgM Capture ELISA kit (MAC-ELISA) for detection of antidengue virus immunoglobulin M (IgM) antibody is based on the capture of dengue virus-specific IgM antibodies in serum or whole blood dried on filter paper strips[18].

As far as the seasonal variation was concerned most cases 34% and 34.1% were seen in the months of October to December and July to September respectively . The period from March found only 9.5% of cases and the summer month's incidence was 21.4% Transovarial transmission of dengue virus is a crucial etiological phenomenon responsible for persistence of virus during inter-epidemic period of the disease. The role of environmental factors in infectious diseases is well-known. In most countries, dengue epidemics are reported to occur, during the warm, humid, and rainy seasons, which favour abundant mosquito growth and shorten the extrinsic incubation period as well[19,20,21]Transmission of dengue increases during monsoon.Chakravati et all pointed out that the outbreak coincided mainly with the post monsoon period of subnormal rainfall. The difference between serologically positive cases as compared to serologically negative ones in post monsoon period was significantly higher ($p < 0.001$). The difference in the rainfall and temperature between three seasonal periods was significant ($p < 0.05$)[22]Nishant et all study showed that Indigenous cases from Delhi appeared from third week of June. Maximum number of specimens was received in September 39.9% followed by October 30.4% The maximum cases diagnosed were also in September 46.0% followed by October 26.4% .Percentage positives of the suspected cases peaked in September and August (44.8% and 44.2%, respectively) dengue virus need clear water to breed.[23].In a study by Wonkoon et all in Sisaket Thailand showed There were more Ae. aegypti larvae per household than Ae. albopictus larvae in the winter and rainy seasons. More Aedes larvae per household were found in the rainy season than in the winter and summer seasons. Relative humidity at a lag of one month and rainy days in the current month were significant predictors of dengue incidence in Sisaket[24].The male to female ratio was found to 1.7:1,inMuhammad Murtuzaet al study in tertiary hospital in Karnataka[2]the study conducted by Ashwini Kumar et al reveals similar ratio 1.8:1.[26]

81.7% of the patients presented within 1-5 days of fever. Health care proximity and availability makes these patients come to hospital for the febrile illness. Dengue awareness created by health services and media also plays a role. The classical symptoms of severe body pain fever and rash are enough to make most seek medical help. All patients i.e. 100 % had fever and 64.3% had myalgia. Rash was seen in 46% of the patients. Petechiea was seen in 30.2 % of the patients. Small number had significant complications like renal failure 3.25 and 4% had ARDS. These finds were also seen in other clinical studies of dengue. A Study of Clinical Profile of Dengue Fever in Kollam, Kerala found presenting symptoms were: fever (96.8%), headache (77.2%), bleeding (15.2%), skin rash (13.2%), sore throat (5.2%), The major physical findings noted included positive tourniquet test (33.67%), hepatomegaly (17.6%), pleural effusion (13.2%) and ascites (12%). The most frequent abnormal laboratory findings included haemoconcentration (27.8%) and severe thrombocytopenia[25] .A study by Ashwin Kumar et al in Karnataka described fever in 99.1%, followed by myalgia 64.6%, vomiting 47.6%, headache 47.6% and abdominal pain 37.6%. The most common haemorrhagic manifestation was petechiae 67.2%. Out of 14.1% patients who developed clinical complications, 33.3% had ARDS and 30.3% had pleural effusion. They has a higher incidence of ARDS[26].

AS far as laboratory parameters were concerned only 4.8% had Hb below 10 and PCV less than 30 in 4. Unless there is clinical bleeding Hb does don't fall in dengue. Kaur-Ramndeep et al in their study on haematological changes in dengue described Hb levels low due to disseminated intravascular coagulation , where chemicals responsible for clotting are used up and lead to risk of severe bleeding or the toxic effects on platelets in the presence and absence of acute and convalescent patient serum. Bleeding was significantly related to severe thrombocytopenia. Most of the patients who had bleeding had a decrease haematocrit[27].

In our study 83.3% had a leucocyte count less than 4000. 75.4% had a platelet count less than 1 lakh and 31% between 1 to 1.5 lakh, and 11.1% less than 50000. 96% showed a rise in SGOT and 85.7 %rise in SGPT. Leukopenia, thrombocytopenia, and haemorrhagic diathesis are the characteristic hematologic findings in dengue virus infection. Leukopenia appears early in the course of illness and is thought to occur as a direct effect of dengue virus on the bone marrow[28]. Dengue also causes a transient decrease in maturation of erythroid precursors,[31] however because of the long half-life of the red cells, dengue does not cause severe anaemia in infected individuals. Cause of thrombocytopenia is multifactorial. Both bone marrow suppression and platelet destruction contribute to thrombocytopenia, with latter playing a more important role.A study by rahulunnikrishnan et al at presentation, thrombocytopenia (platelet count <150 K/µL) (77.4%) was the major haematological abnormality followed by leukopenia (total white blood cell count <4 K/µL) (52.8%) and anaemia) (13.2%). Hepatic derangement, as signified by the elevation of SGOT (92.5%) and SGPT (71.7%). Renal dysfunction was observed in (15.1%[2]□)Our study also showed similarities in hepatic derangements.

A study in a tertiary hospital in Karnataka by Muhammad Murtuza found 80.29% patients had platelet count less than 100000 [2]. Leukopenia was observed in same study

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in 43.83% of patients whereas Prafull Dutta et al, reported 30.0% of patients presented with leukopenia.[30] Our study showed a higher incidence of leucopenia. Liver enzymes like AST was found in 1/4th of study population in Muhammad Murtuzas study whereas PrafullaDutta et al, reported in 1/3rd of study population and ALT were in 1/4th of study population whereas other study shows half of the patients.[14] So AST and ALT was less affected in the region of central Karnataka study by Prafulla Dutta.

Mortality in our study was 2.4 % ( 3 patients ) .Two patients had presented late with features of hypotension ,renal failure and shock. One patient developed ARDS after admission and died. Mortality from complications of dengue may be as high as 20% but drops to less than 1% if diagnosed early and properly managed [1,32].Manjunath et al [33] had a mortality rate of 8.6 % while Rajesh Deshwal et all [34] had a mortality of .77% and Ahmed et all [35] had a mortality of 3%. The low mortality in our study 2.4 % could be because of early diagnosis based on NS1 Ag assay , early admission and prompt management

**Limitations of the study**

Since this was retrospective study, reliance was more on hospital records available in the case papers. Many patients were discharged within 2-3 days of admission and were lost to follow up. Convalescent sera could not be obtained for testing.

**Conclusion**

In this study we found that NS1 Ag assay is a very useful tool to suspect dengue fever in patients who present early with clinical features suggestive of dengue fever. Early admission of suspected cases along with proper monitoring and prompt management helps to reduce complications and improve survival rates .A combination of NS1 Ag and antibody analysis covers all stage of infection. The simplicity, cost effectiveness and easy availability of these tests are added benefits. This study substantiates that in comparison to MAC-ELISA, NS1 Ag assay is an effective tool for early diagnosis of DV infection, especially within the first four days of illness. Early detection of DHF by NS1 assay can help in early confirmation and management of this vulnerable group.

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