Placental umbilical cord whole blood transfusion to combat anemia in the background of tuberculosis and emaciation and its potential role as an immuno-adjuvant therapy for the under-resourced people of the world

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Summary

Tuberculosis causes approximately 1.5 billion latent infections, 8 million new clinical cases, and 3 million deaths annually, making it the most prevalent infectious disease in the world. Anemia and malnutrition are essential comorbidities with tuberculosis.

Cord blood, because of its rich mix of fetal and adult hemoglobin, high platelet and WBC counts, and a plasma filled with cytokine and growth factors, as well as its hypo-antigenic nature and altered metabolic profile, has all the potential of a real and safe alternative to adult blood transfusion.

We transfused 106 units (48 ml-148 ml mean 81 ml ± 6.6 ml SD, median 82 ml, mean packed cell volume 49.4 ± 3.1 SD, mean percent hemoglobin concentration 16.3 g/dl ± 1.7 g/dl SD) of placental umbilical cord whole blood (from 1 April 1999 to 1st 2005) after lower uterine cesarean section from consenting mothers to 21 informed consenting patients with tuberculosis who had percent plasma hemoglobin of 8 g/dl or less. After collection, the blood was immediately transfused following the standard adult blood transfusion protocol. Each case was passed through the institutional ethical committee. The patients received 2-21 units of freshly collected placental umbilical cord blood without encountering any clinical, immunological or non-immunological reactions. Three days after completion of the placental umbilical cord blood transfusion, the peripheral blood hematopoietic stem cell (CD34) estimation revealed a rise from the pretransfusion base level (.09%), varying from 2.99% to 33%, which returned to base level in 66.66% at the three-month CD34 re-estimation, without provoking any clinical graft vs host reaction in any of the patients.

Key words: Placental umbilical cord blood transfusion; Safe; Tuberculosis.

Introduction

Tuberculosis (TB) represents an important worldwide health hazard. It has been reported by the World Health Organization (WHO) that one person in the world becomes infected every second, and that one third of the entire population of the world is now infected. The WHO also estimates that in the next decade, 300 million more people will be infected, 90 million people will develop the disease, and 30 million people will die from it [1]. Among those over five years of age, tuberculosis kills more people than AIDS, malaria, diarrhea, leprosy, and all other tropical diseases combined. The tragedy of this situation is that treating tuberculosis today is one of the most efficacious and cost-effective of all health interventions. The estimated annual number of deaths was 3 to 4 million by the year 2004 [2]. Patients with persistent pulmonary infections from mycobacterial diseases present a difficult clinical challenge. These individuals typically have poor pulmonary function, malnutrition, and other comorbidities, and few guidelines exist regarding optimal therapy. The prevalence of tuberculosis (TB) infection and clinical disease varies widely in different age groups. However, among children in household contact with adult patients, it is higher than in the general population, and the risk is significantly increased by contact with sputum-positive adults [3]. The nutritional history of patients with TB has revealed that they have higher levels of anorexia, vomiting, nausea and diarrhea. Consequently, both male and female TB patients suffer from considerable malnourishment. It has been recommended that these patients should receive nutritional support during their treatment, with studies of the exact nutritional deficiencies at the micronutrient level and their effect on the immune system being required [4]. Directly observed treatment-short course (DOTS) has been a successful strategy in the global control of TB in adults. However, there are uncertainties about TB in extremes of ages, i.e., in the pediatric and geriatric age groups, as well as in adults who receive steroids and other immunosuppresives, or in the case of uncontrolled diabetes or nutritionally deprived patients in the under-resourced regions of the world. In such cases, patients can present to a physician with vague clinical symptoms, unreliable tuberculin tests or TB score charts, non-specific hematological, biochemical or radiological evidence, difficulty in sputum expectoration and non-availability or ill-affordability of specialized tests [5].

Anemia in tuberculosis is a serious comorbidity, which

The work was supported by a research grant from the Dept. of Science and Technology, Govt. of West Bengal.

Revised manuscript accepted for publication November 12, 2005

is caused by various factors, for instance, hemoptysis in cases of pulmonary Koch's in advanced stages. It could also occur as a result of lack of proper nutrition and micronutrients in the diet, or coexistent helminthiasis, and/or other coexistent diseases like HIV and/or other preexisting or compounding gastrointestinal problems which alter the available iron stores or reserves or cause bone marrow dysfunction. In the Indian subcontinent, malnutrition and anemia,weakness with evening rise of temperature and an emaciated look are a quite typical presentation in persons with tuberculosis in the rural and semi-urban areas, who report to state government hospitals for free medicine and other essential drug support.

My team of doctors has been successfully transfusing placental cord whole blood, which is rich in fetal hemoglobin content as well as cytokine and growth factors, as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any etiology [6]. The placenta, or afterbirth, is discarded routinely everywhere in the world (in India alone, there are more than 20 million placentae produced as afterbirth every year), and is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated or incinerated. Centers of excellence in the Western developed world have been working on the use of a tiny microscopic fraction of cord blood, i.e., CD 34 stem cells only (.01% of the nucleated cells of placental blood). Whether fetal hemoglobin-rich placental umbilical cord whole blood (which has the potential to carry more oxygen to the tissue vol/vol than adult blood, if collected aseptically after the birth of a healthy newborn at or near term) could be an emergency and safe substitute for adult whole blood in case of tuberculosis victims with percent hemoglobin concentration of less than 8 g, was the main idea behind the present study.

Material and Methods

This is a government hospital-based study conducted in Calcutta (India), where most poor patients are admitted to receive free treatment. This study included marginalized persons, i.e., homeless persons, alcoholics, migrants, drug abusers, landless laborers and the poor from any strata of society. We enrolled patients from our hospital who were suffering from anemia, tuberculosis, emaciation and who could not buy erythropoietin or arrange for fresh whole blood or concentrated RBCs for cord blood transfusion. All enrolled cases gave proper informed consent and were reviewed by the institution-based ethical committee.

Human placental umbilical cord blood (106 U) was collected from consenting mothers aseptically after lower uterine casarean section under general or regional anesthesia. The volume varied from 48 ml-148 ml (mean 81 ml + 6.6 ml SD, median 82 ml) with a mean packed cell volume of 49.4 ± 3.1 SD, and mean percent hemoglobin concentration of 16.3 g/dl ± 1.7 g/dl SD. After collection the blood was immediately tested and transfused or preserved in the refrigerator and transfused within 72 hours of collection if no recipient was available at that time. In case of gross prematurity or dysmaturity, or if the projected weight of the fetus was less than 2 kg, or if there was any specific disease affecting the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anesthetist verified the stable physical condition of the mother. It was only then that the obstetrician made the decision to proceed with the umbilical cord blood collection. Immediately, the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14 ml anticoagulant citrate phosphate dextrose adenine solution) which was used for the purpose of collection. When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1-4°C after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, malaria, fungus and bacteria, as per standard blood transfusion protocol, on which we have reported earlier [7, 8]. The collected cord whole blood was transfused immediately or at the most within three days of collection to a patient with anemia, after grouping, cross-matching and following the standard adult blood transfusion WHO guideline for transfusion, and simultaneously, strictly adhering to the institutional ethical committee's instructions and the patient consent protocol. Pretransfusion and three days after the transfusion, blood was drawn from consenting patients for peripheral blood hematopoietic stem cell estimation (CD34) by flow analysis cytometry as per standard protocol at Ranbaxy Laboratory. This procedure was repeated after three months for comparison.

Results and Analysis

As mentioned earlier, tuberculosis is one of the most important chronic diseases in the world and a leading infectious cause for millions of deaths annually [9]. Varying degrees of anemia are very prevalent in TB patients, which could be due to poor nutrition, coexistent diseases like helminthiasis or drug impact on the immune system (including the bone marrow), and poor red cell survival.

The rationale for the treatment of anemia in chronic diseases is based on two principles. First, anemia can be generally deleterious in itself, requiring a compensatory increase in cardiac output in order to maintain systemic oxygen delivery; second, anemia is associated with a poorer prognosis in a variety of conditions. Thus, moderate anemia warrants correction. Blood transfusions are widely used as a rapid and effective therapeutic intervention in anemia. Transfusions are particularly helpful in the context of either severe anemia (in which the hemoglobin is less than 8.0 g/dl) or life-threatening anemia (in which the hemoglobin is less than 6.5 g/dl), particularly when the condition is aggravated by complications that involve bleeding.

We transfused 106 units of freshly collected placental umbilical cord whole blood within 72 hours of collection from consenting patients undergoing lower uterine cesarean section maintaining standard WHO specified blood transfusion norms for our country. Each case has been followed-up to date.

Twenty-one patients with anemia (8 g/dl or less) in the background of tuberculosis were included in this study. Sixteen cases were suffering from pulmonary Koch's, and presented with cavitation in four cases. The remaining five cases had extra-pulmonary Koch's involvement, i.e., intestinal Koch's involvement was detected in four cases and skin involvement in one case.

The criteria for clinical diagnosis of TB were typical clinical features like loss of weight, evening rise of temperature, weakness and other constitutional symptoms depending on the primary involvement of the organ. For assessment of the Koch's status, primary or reactivation and the background, X-rays were taken, and usual tests like the Mantoux test, hemoglobin, total WBC count, differential count of WBCs and erythrocyte sedimentation rate were done. However, in extra-pulmonary and non hemoptosis, silent presentation of suspected Koch's infection, Elisa TB IgA, IgM, IgG and screening for HIV 1 and 2 were done routinely in the younger age group. In case of clinical confusion, adenine deaminase, gamma interferon, fine needle aspiration cytology and biopsy were used as supportive investigation. In two cases, we had to study DNA (by PCR) via ascitic fluid and also from serum for confirmation. In the pulmonary presentation group, in addition to Koch's reactivation, two cases had HIV and four cases had cancer. The group included 13 females and eight males with their ages varying from 18 to 74 years. A total of 106 units of freshly collected blood was transfused to these patients without encountering a single case of immunological or non immunological reaction. The patients received 2-21 U of blood with 8 U in a row in one case to combat anemia due to hemoptysis-induced blood loss. All the patients who received this blood had positive clinical responses like less weakness, a sense of well being and weight gain, which was quite obvious in patients who received more than three units of blood (16 cases).

What is interesting, and we have not been able to trace any similar phenomenon in the published medical literature, was the rise in the peripheral blood CD34 level assessment by flow analysis cytometry done 72 hours after transfusion. This test was repeated after three months in consenting volunteers (Figure 1). The normal CD34 level in peripheral blood is .09%. In the present series of patients suffering from TB with an anemic background, among those who received cord blood, the level of CD34 varied from 2.99 to 33%. This returned to individual base levels in 66.66% of the cases, at the three-month CD34 re-estimation. The point at issue is why did this rise in CD34 level take place, and why did it vary from individual to individual without provoking any clinical graft vs host reaction. No patient in the present series (HLA- and sex-randomized) received any specific immunosuppressive drug therapy apart from the antitubercular drug. One possible answer could be the hypo antigenic cord blood and immune mosaic condition in tuberculosis. Another could be that freshly collected cord blood contains growth stimulating cytokines, which may have an impact on the hosts' bone marrow or any other specific system.

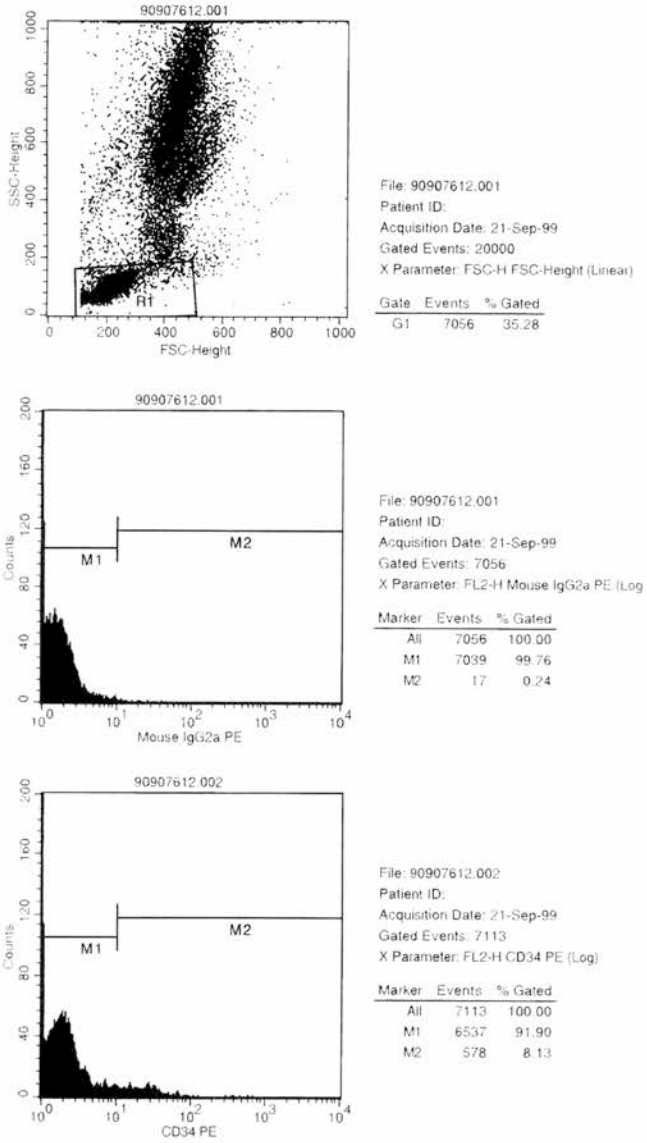

Figure 1. — Flow analysis cytometry report (one case) of the peripheral blood showed a 8.13% CD34 level collected from the peripheral blood 72 hours after an ABO/Rh group cross-matched cord blood transfusion.

Discussion

In the developing world as a whole and in India in particular, it is common knowledge to a physician treating tuberculosis, that nutritional deterioration may help in activating the latent or subclinical form of TB. Protein malnutrition has been identified as an important risk factor for the predisposition to intracellular infections leading to destabilization of the clinical condition. There is a strong association between protein malnutrition and impairment of a range of immune functions, principally those mediated by T lymphocytes, which are known to be essential for resistance to tuberculosis.

Another characteristic is anemia resulting from chronic disease, as seen in TB. Here, there are disturbances of iron homeostasis with increased uptake and retention of iron within cells of the reticuloendothelial system. This leads to a diversion of iron from the circulation into storage sites of the reticuloendothelial system, subsequently limiting the availability of iron for erythroid progenitor cells, and causing iron-restricted erythropoiesis. In mice that are injected with the proinflammatory cytokines interleukin (IL)-1 and tumor necrosis factor α (TNF-α), both hypoferremia and anemia develop [10]. In chronic inflammation the acquisition of iron by macrophages most prominently takes place through erythrophagocytosis [11] and the transmembrane import of ferrous iron by the protein divalent metal transporter 1 (DMT1) [12]. Interferon-γ (IFN), lipopolysaccharide, and TNF-α up-regulate the expression of DMT1, with an increased uptake of iron into activated macrophages [13]. The identification of hepcidin, an iron-regulated acute-phase protein composed of 25 amino acids, has helped to shed light on the relationship of the immune response to iron homeostasis and anemia in chronic disease. Hepcidin expression is induced by lipopolysaccharide and IL-6 and is inhibited by TNF-α [14].

In India tuberculosis remains the leading infectious cause of death, killing close to 500,000 people a year. India has far more cases of TB than any other country in the world - about 2 million new cases each year [15], and accounts for nearly one-third of prevalent cases globally. The Indian TB-control program is now one of the largest public health programs worldwide. The program has been remarkably successful, although it still faces many challenges. Direct health benefits to date include the treatment of 1.4 million patients with TB and prevention of more than 200,000 deaths [16].

Primary tuberculosis, a self-limited, mild pneumonic illness that often goes undiagnosed, may develop in a subgroup of infected persons - nutritionally deprived or immunocompromised individuals, or in very young pediatric patients. During this illness, bacillemia and seeding of other organs may occur, setting the stage for subsequent reactivation in extra pulmonary sites. In the USA, one investigator has suggested that in about 5% of persons the infection progresses from a latent form to active disease within two years after infection, and an additional 5% have active disease at some later point in their lives. Although the majority of cases of active TB are thought to arise from a reactivation of latent infection, exogenous re-infection with a second strain of *M. tuberculosis* can occur, particularly in profoundly immunocompromised persons and in those heavily exposed to new bacilli. Nucleic acid technology provides rapid, specific, and sensitive diagnostic tests and rapid detection of drug resistance [17-19]. Important clinical signs of pulmonary TB include cough, pleuritic pain, and hemoptysis. Though the lung is involved in 80% or more in the USA, extra pulmonary sites of disease are also seen, i.e., the lymph nodes, pleura, bones or joints, the genitourinary system, central nervous system, abdomen and pericardium, and in rare cases, virtually any other organ. Diagnostic standards and classification of TB in adults and children have been widely discussed in the medical literature [20]. However, the clinical signs of pulmonary tuberculosis are more varied and less specific in persons with HIV infection [21].

Recent advances in the treatment of TB like combination therapy have revolutionized the outcome of the disease. But in the under-resourced world, the real problems are hunger and malnutrition, which have a very important role in reactivation of the disease. Hence, the presentation becomes uniquely compounded and the responsibility of treatment and the outcome become less positive.

For example, anemia in chronic diseases like tuberculosis is the result of acute or chronic immune activation due to inflammation. This anemia is the second most prevalent variety after anemia caused by iron deficiency [22]. In severe anemia when the hemoglobin level is 8 g/dl or less, we often decide to give a packed cell transfusion slowly. The 21 patients in our hospital who volunteered for the cord blood transfusion and received a total of 106 units, did not encounter a single case of immunological or non immunological reaction. We consider this to be very positive news.

What is intriguing is the rise in peripheral blood CD34 level after cord blood transfusion as seen in the flow analysis cytometry report. The reason for the transient rise of hematopoietic stem cells in the peripheral blood in HLA-randomized recipients without any immunosuppressive support and without provoking clinical graft vs host reaction, still remains a mystery.

However, we can venture some probable explanations. The placenta has a unique microenvironment and its sensitization impact on cord blood cells may have a role in transient transplantation impact on the host system. One very important factor, apart from intrinsic differences, is the fact that hematopoietic stem (HS) cells in umbilical cord blood (UCB) cells have had a different set of microenvironmental exposures compared to those of adult marrow or peripheral blood stem (PBS) cells. An example of differences between sources are some of the observed changes in HS cell cycle status, gene expression and the adhesive and invasive properties induced by mobilization procedures used to generate PBS cells, e.g., granulocyte colony stimulation factor (G-CSF). The placenta is a complex organ that regulates maternofetal interactions. Many cytokines that can influence lymphohematopoietic development, e.g., G-CSF, c-kit ligand (stem cell factor [SCF]), granulocyte macrophage colony stimulated factor (GM-CSF), IL-15, and others, are produced by the placenta. Production of G-CSF by the placenta may be especially relevant to UCB transfusion. G-CSF is produced both by the maternal decidua and fetal chorionic villi and enters the fetal circulation by a process that does not require a functional G-CSF receptor. G-CSF from the mother probably does not enter the fetal circulation, as administration of recombinant

human G-CSF (rhG-CSF) to pregnant macaques did not result in detectable rhG-CSF in the fetuses [23]. The function of placental G-CSF production is unknown; however, it may serve as an immunoregulator that protects the mother and fetus from each other's allogeneic immune systems. G-CSF inhibits the ability of placental mononuclear cells to mediate cytotoxicity against allogeneic targets including choriocarcinoma cells.

Finally, though precisely not clear as yet, the functional hypoantigenecity of freshly collected and immediately transfused cord blood antigen with its complex cytokine interaction, may have a role in immune selective masking, i.e., immune mosaicism, in anemic patients with tuberculosis who have immunosuppression either due to drugs, the chronic nature of the disease, malnutrition with helminthiasis, or other associated factors like the impact of growth factors or selective cytokine impact of the cord blood on the bone marrow of the recipient. All the patients irrespective of their background tolerated the procedure well and there was a sense of well being in most of the patients along with weight gain in 85.5%. There was no clinical graft vs host reaction or any other immunological or non immunological reaction among the transfusion recipients in this present study and in the follow-up so far.

Our group of researchers is working on the problem of fetal cell or tissue transplant in the adult system [24-28]. We have also worked on the use of umbilical cord whole blood transfusion as an alternative to adult whole blood transfusion from the pediatric to the geriatric age group for different indications since 1 April 1999. We are of the opinion that cord blood collected aseptically from the placenta of consenting mothers after the birth of a healthy baby has all the potential of an effective therapeutic adjuvant for TB patients with anemia in the underprivileged world. Cord blood is protected from infection as a result of nature's finest biological sieve, i.e., the placenta, and contains 60-80% fetal hemoglobin (which can carry 60% more hemoglobin than adult hemoglobin). Moreover, it has a high WBC and platelet content, is hypoantigenic in nature, and has an altered metabolic profile. It may also have the potential to convert TH2 responses to TH1 responses due to its rich cytokine and growth factor content which may have a role in immune response modification.

Conclusion

Tuberculosis causes approximately 1.5 billion latent infections, 8 million new clinical cases and millions of deaths annually, which makes it the most prevalent infectious disease in the world [29]. The incidence of TB is unusually high among malnourished people, including the elderly, the homeless, alcoholics, drug abusers, and human immunodeficiency virus-infected individuals. The detrimental effects of nutritional deficiencies on TB could result from alterations in T lymphocytes and macrophage functional regulation, which are the major cell types mediating antimycobacterial immunity [30]. Cytokines play a central role in mediating antimycobacterial immunity. IL-2 is required to initiate and amplify immune responses. IFN-γ and TNF-α are important macrophage-activating cytokines and crucial in the immune response to tuberculosis [31]. Other advances in our understanding of the pathophysiology of anemia in chronic diseases like tuberculosis – disturbances of iron homeostasis, impaired proliferation of erythroid progenitor cells, and a blunted erythropoietin response to anemia – have made possible the emergence of new therapeutic strategies. These include treatment of the underlying disease and the use of erythropoietic agents, iron, or blood transfusions. Despite inadequate funding resources, much effort is being devoted to both approaches, involving a multidisciplinary approach from diverse disciplines such as molecular biology, social anthropology, and health economics.

Umbilical cord blood is now accepted as an alternative source for hematopoietic stem cells for transplantation, especially in children. Unrelated UCB offers many practical advantages as an alternative source of stem cells, including: 1) easy availability without risk for mothers or donors; 2) less possibility of infection, particularly cytomegalovirus; 3) documented reduced risk of GVHD, with easy HLA-matching criteria for donor-recipient selection, and 4) UCB banks have been established for related and unrelated UCB transfusions with about 100,000 units currently available [32]. However, so far, all centers in the world discard the precious umbilical cord blood after separating .01% of stem cells from it. We are the only researchers in the world who are working on the use of fetal hemoglobin and cytokine rich umbilical cord whole blood as an alternative source of blood, and transfusing this umbilical cord whole blood to our volunteers suffering from TB and anemia since 1999.

The clinical improvement as a result of cord blood transfusion and the transient rise in the peripheral blood CD34 level, stimulates us to think of the probable adjuvant immunopotentiating role or immunotherapeutic impact on the hosts' suppressed immune system which had been caused by malnutrition, chronic disease or even drug impact. Hepatotoxicity occurs with isoniazid, rifampicin, pyrazinamide and ethionamide. Risk factors include old age, malnutrition and high alcohol consumption. If cord blood has an adjuvant immunotherapeutic impact, it may play a positive role in negating immunosuppression in TB patients.

In this preliminary communication after six-years of follow-up, we wish to note that the use of cord blood transfusion in TB patients with anemia is not only safe, but the unique phenomenon of transient spontaneous transplant impact of the CD34 cells, as seen in the peripheral blood after cord blood transfusion in HLA-randomized recipients, without the support of immunosuppressives and without provoking clinical graft vs host reaction, is an interesting positive finding. This has never been cited before in published medical literature and we hope further studies will be undertaken.

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