Immunoscintigraphy with ^{99m}Tc -Besilesomab in Aid to Fusariosis Detection in Patients with Relapsed Acute Myeloid Leukemia (AML): A Case Report and Literature Review

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Abstract: The aim of this article is to emphasize the importance of nuclear medicine in fungal infection characterization through a case report. Case: patient female, with AML-M2, hospitalized with fever, dyspnea, nausea, myasthenia, abdominal pain and diarrhea. In the physical examination exacerbated lesion in abdominal wall and subcutaneous nodules in upper and lower limbs. The computed tomography of the chest evinced area of attenuation in ground-glass opacities in the middle lobe, tending to consolidation and bilateral nodular opacities with ground-glass halo which suggested an inflammatory/infectious process of fungal etiology. Immunoscintigraphy with ^{99m}Tc -besilesomab (Scintimun[®]) identified focal infectious processes in activity, scattered over cutaneous and subcutaneous tissues, predominantly in extremities. In blood culture test, there was a growth of *Fusarium* sp. There is a difficulty in the diagnosis of fungal infections, both in clinical and imaging methods. There is no specific method to reaching the target. In addition, fungal diseases can be divided into focally localized or disseminated infections. There is a greater difficulty in the diagnosis of immunocompromised individuals due to the deficiency of basic defense mechanisms. Scintimun[®] (scintigraphy with besilesomab) is a useful tool of nuclear medicine in the diagnosis of infectious and inflammatory diseases to allow the image of the whole-body and *in vivo* detection of early pathological and physiological phenomena, even before anatomical alterations occur.

Key words: Immunoscintigraphy, besilesomab, nuclear medicine, fusariosis, fungal infections, immunocompromised.

1. Introduction

Immunocompromised patients are more liable to a risk of developing infections due to the deficiency of basic defense mechanisms [1]. Granulocytopenia with cellular defense decrease, mediated by T-lymphocytes and the defect in number and function of macrophages would be responsible for this occurrence. Fungi are associated with a broad spectrum of diseases in human beings that range from cutaneous lesions and self-limited acute pulmonary infections to severe and

potentially fatal infections. As the number of immunocompromised individual population increases, secondarily to cancers, chemotherapy, organ transplants, and autoimmune diseases, there is also an increase of fungal diseases [1].

The defense of fungal infections in the organism takes place through two mechanisms: innate and adaptive. In the first innate case, there is a phagocytosis with direct destruction of the pathogen through phagocytic cells, such as monocytes, macrophages and neutrophils, as well as cells that are not phagocytic as epithelial and endothelial are. In the second adaptive mechanism, there is a fungi uptake by

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dendritic cells, inducing maturation of these cells and promoting the differentiation of native T cells into T helper subtypes [2].

To achieve optimal activation of specific adaptive immune response, it is necessary to activate the detection mechanism of pathogens by innate immune system first [2]. Nevertheless, in some fungal infections, several potent regulatory factors are produced from host inflammatory response, in order to mask or subvert the pathogen detection systems. The model called “Trojan horse” suggests that there is a replication, transference of genetic material and potential expulsion of yeast debris from macrophages, remaining the latent infection, without triggering the inflammatory processes [2].

Although the epithelial cells do not have the role of antigen-presenting cells, they may be important in immune response through receptors stimulation, initiating and amplifying the response of T helper cells, which stimulates interleukins and therefore phagocytic cells [3], including granulocytes, which there may be besilesomab (Scintimun®) uptake in peripheral fungal infections.

Pulmonary complications represent the major cause of morbidity and mortality in an immunocompromised host [4, 5]. Regardless of the cause of immunosuppression, bacterial, viral and fungal, infections are the most frequent in pulmonary impairment. Symptoms such as dyspnea and hypoxemia in immunocompromised patients indicate the need of fungal screening [5]. Among fungal infections, aspergillosis (*Aspergillus* sp.) is the most common with incidence of 1~9% and mortality of 55~92%, followed by candidiasis (*Candida* sp) [6]. Fusariosis (*Fusarium* sp) as an emerging pathogen, being possible to be the third cause reason of fungal infection in some centers [7, 8].

Fungi of the genus *Fusarium* sp are commonly found in soil or as plant pathogens, rarely affecting patients, which in these cases, are frequently immunocompromised by hematologic neoplasia [9].

Infection by *Fusarium* sp. can be localized, focally invasive or disseminated. Fusariosis in its disseminated form is a rare infection of difficult diagnosis suspicion, even with treatment, exhibit most of times, complications of high morbidity and mortality [10, 11]. The diagnostic delay increases the risk of death in an immunocompromised host.

Nuclear medicine plays an important role in the characterization of diagnosis, and evolution of infectious and inflammatory processes. Examples are immunoscintigraphy with anti-granulocyte monoclonal antibodies, being besilesomab (Scintimun®) is the most known one, scintigraphy with labeled leukocytes such as: ¹¹¹In-oxine (¹¹¹Indium-8-hydroxyquinoline) [12] ^{99m}Tc-SC (technetium-99 metastable-sulfur colloid) [13] and ^{99m}Tc-HMPAO (technetium-99 metastable-hexamethylpropylene amine oxime) [14], scintigraphy with ⁶⁷Ga (gallium-67 citrate) [15], positron emission tomography scan (PET) with ¹⁸F-FDG (18F-fluorodeoxyglucose) [16], and scintigraphy with anti-fungal agents, such as fluconazole labeled with ^{99m}Tc (metastable technetium), being the latter an experimental drug [17].

Scintimun® (besilesomab) labeled with metastable technetium ^{99m}Tc (technetium) is an anti-granulocyte monoclonal antibody produced from murine cells (IgG1k BW 250/183), which binds to the NCA-95(non-specific cross-reacting antigen 95), also referred to as CD66b and CEACAM8 [17] as an epitope expressed on the membrane of granulocytes cells and granulocyte precursors suitable for the evaluation of infectious and inflammatory processes, both in soft tissues regions and bone regions. The first approach on in vivo labeling with murine antibody ^{99m}Tc-besilesomab (Scintimun®) was in 1992. In January 2010, marketing authorization of Scintimun® in all European countries by the EMEA (European Medicines Agency) was granted [18]. Since then, it has been used successfully in the study of subacute infectious endocarditis [19], lung abscesses [20], diabetic and septic foot infections [21], loosening of

hip and knee prostheses [22, 23], and osteomyelitis [24], despite the decreased sensitivity in spinal infections, for being a physiological uptake site, besides important limitation in inflammatory bowel diseases [25], due to non-specific intestinal absorption of this drug, producing questionable.

The clinical case described indicates the use of ^{99m}Tc -besilesomab (Scintimun[®]) in the diagnosis of a disseminated fungal infection by *Fusarium* sp., being unprecedented in the literature.

2. Case Report

Female patient, 32 years old, diagnosed with AML-M2 (relapsed acute myeloid leukemia) a year after receiving consolidation chemotherapy with Cytarabine for bone marrow transplant from HLA compatible sibling. She was hospitalized with fever, dyspnea, nausea, myasthenia, abdominal pain and diarrhea. In the physical examination port-a-cath do not have phlogistic signs, ulcerated injury in abdominal wall, and subcutaneous nodules in upper and lower limbs. The CT (computed tomography) of the chest demonstrated extensive area of ground-glass

opacities in the middle lobe, tending to consolidation and sparse and bilateral nodular opacities with ground-glass halo and distribution preferably in vascular extremities, an aspect which suggested an inflammatory/infectious process of possible fungal etiology. Immunoscintigraphy identified infectious processes in activity, scattered over cutaneous and subcutaneous tissues, predominantly in extremities of upper and lower limbs. It was not possible to identify the radiotracer uptake in the lung fields, probably due to granulocytopenia in this site. In blood culture there was growth of *Fusarium* sp. after 5 h of incubation. The patient was treated with Voriconazole for 35 days, producing involution of lesions, but with worsening of medical condition, progressing into death from febrile neutropenia (Figs 1-4).

3. Discussion

Besilesomab (Scintimun[®]) has 86% sensitivity, 78% specificity, and 81% diagnostic accuracy in the detection of infectious processes [26]. It is administered about 40~800 megabecquerel MBq of ^{99m}Tc -besilesomab, which corresponds to 0.25~1.0 mg

Fig. 1 Computed tomography showing extensive area of ground-glass opacities in the middle lobe, tending to consolidation and sparse and bilateral nodular opacities with ground-glass halo and distribution preferably in vascular extremities, an aspect which suggested an inflammatory/infectious process, stressing the possibility of fungal etiology.

Fig. 2 Whole body immunoscintigraphy of 4 h and 24 h after ^{99m}Tc -besilesomab injection showing diffuse lesions in the cutaneous and subcutaneous tissues.

of Scintimun[®] by intravenous administration. The interval between administration of the radiopharmaceutical and image acquisition to reach good relation target/no target ratio is around 4–24 h. Signore A and collaborators [27], 2011, showed that the diagnosis of infection and inflammation with anti-granulocyte monoclonal antibodies (Scintimun[®]) had a higher sensitivity in the same patient than labeled leukocytes ^{99m}Tc -HMPAO (74.8% in the first and 59.0% in the second method) and specificity was slightly lower (71.8% in the first and 79.5% in the second method).

Some studies have shown that only a small percentage of anti-granulocyte monoclonal antibody injected into the patient actually binds directly on the

cell membrane [17, 27]. Most of it will be concentrated by the overflow of the radiopharmaceutical in infected or inflamed sites in a nonspecific way through vascular permeability of endothelium increase.

The best-known anti-granulocyte monoclonal antibodies are besilesomab (Scintimun[®]), fanolesomab (LeuTech[®]), and sulesomab (Leukoscan[®]). Their advantage is their easy handling, it is not necessary an *in vitro* manipulation of white blood cells, and they are not potentially dangerous, from a biological point of view when compared to radiolabeled autologous leukocytes. This technique allows the detection of infection or inflammation throughout the organism at an early stage of the disease, which could be difficult to assess clinically or through other imaging techniques.

Fig. 3 Static images of the upper limbs after 4 h of ^{99m}Tc -besilesomab injection showing lesions predominantly in the extremities.

Some disadvantages would be the high molecular weight, resulting in slower diffusion in the sites of infection or inflammation, and hepatic uptake due to clearance by the reticuloendothelial system [17, 27].

Besilesomab (Scintimun[®]) rarely induces side effects in injected patients. The described side effects are hypotension, hypersensitivity, and the most relevant, induction of HAMA (human anti-murine antibody). If a second injection is performed and the body has produced HAMA, bio-distribution, quality and clinical relevance of these images may be compromised. Thus, it is mandatory to make a HAMA test, available in kit and performed, before the injection

of the radiopharmaceutical [19, 28].

Transient neutropenia, a rare side effect observed in some patients, did not represent any clinical problem or loss to the technical quality of the image. Another disadvantage described in the use of anti-granulocyte monoclonal antibodies was the incidence of serious and potentially fatal cardiopulmonary reactions, despite being very rare [19].

At least theoretically immunogenicity is smaller, the blood clearance is faster and accumulation of the radiopharmaceutical in infected areas is higher, which make the limitations described in the use of besilesomab and other monoclonal antibodies lower [19].

Fig. 4 Static images of the lower limbs after 4 h and 24 h of ^{99m}Tc -besilesomab injection showing lesions predominantly in the extremities.

Scintigraphy with labeled leukocytes is considered the gold standard due to its high specificity, because it only accumulates in inflamed or infected tissues [26-28]. It is widely used for evaluation of osteomyelitis, joint or cardiac prosthesis infection, diabetic foot, fever of indeterminate origin, postoperative abscess, lung infections, endocarditis, inflammatory bowel disease, neurological infections, and infected central venous catheters [27]. It has as a disadvantage with the risks of infection from biological samples for both the operator and the patient. Strict aseptic conditions are necessary for the marking procedure (sterile gloves, cap and mask), marking in

laminar flow or cell isolator, care in order to not damage the leukocytes nor have adherence to the vascular endothelium [28]. The risks of malignant lymphoproliferative disease after administration of ^{99m}Tc -HMPAO are insignificant.

4. Conclusions

Scintigraphy with besilesomab (Scintimun[®]) is a useful tool in the diagnosis of infectious and inflammatory diseases for allowing whole-body images to be made. Immunoscintigraphy has been used successfully to detect several types of infection and inflammation due to its easy handling compared to the

technique with radiolabeled autologous leukocytes. Immunocompromised patients are more likely to develop bacterial, viral, and fungal infections, especially the disseminated ones, due to deficiency of basic defense mechanisms. Diagnosis of fungal infections is still a challenge for medicine. Diagnostic imaging techniques have high sensitivity and low specificity in most cases. Nuclear medicine examinations, however, provide *in vivo* detection of early pathological and physiological phenomena, even before anatomical changes occur.

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