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Case report

Metagenomic next-generation sequencing (mNGS) for diagnostically challenging infectious diseases in patients with acute leukemia

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ABSTRACT

This report shows the contribution of next-generation metagenomic sequencing (mNGS) as an alternative to challenging diagnostic infection in immunosuppressed individuals. Herein, we report three acute leukemia patients who developed severe invasive infections due to different etiologies: fungi, viruses, and protozoa. mNGS improved the diagnosis of the infections and provided the opportunity for adequate therapy. The mNGS is a hypothesis-free diagnostic platform, increasing potential in challenging diseases in hematological patients due to the extended diagnostic panel and the expedite access to the result.

Introduction

Infections remain the most important cause of death in an oncohematological settings, and the spectrum of pathogens is vast and increases concomitantly with immunosuppression.[1] Empirical therapies are frequently indicated but with several limitations: overuse of antimicrobials, emergency of antimicrobial resistance, adverse events, and risk of failure. The delay in diagnosis implies a delay in starting appropriate therapy and worse outcomes. Traditional tests based on cultures, biological methods, and serological tests are time-consuming and sometimes are not sufficient to diagnose several infectious complications on hematological patients. Therefore, alternative methodologies are needed for a prompt and correct diagnosis.[2,3]

Next-generation metagenomic sequencing (mNGS) has been applied in several scenarios: sepsis, meningitis and encephalitis, bloodstream infection, pneumonia, and others. In these situations, routine microbiologic testing is frequently insufficient to detect uncommon pathogens, making sequencing an attractive approach for the detection of a high number of pathogens in a single platform. This characteristic also con-

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tributes to significantly decrease the turnaround time for the result, helping to expedite initiation of an adequate treatment and to have a better outcome.[2,3]

Case reports

Case 1

A 38-year-old female with acute myeloid leukemia achieved complete response following induction of remission (cytarabine and daunorubicin) and two consolidations with high dose cytarabine. She developed febrile neutropenia in all chemotherapy cycles without microbiological documentation. Antifungal prophylaxis with fluconazole was given in the first and second cycles. Three months after the last consolidation cycle, she developed fever and malaise. No findings in the clinical exam were noted, and blood cultures were negative. An abdominal CT scan documented solid lesions in the liver. Voriconazole was started to cover chronic disseminated candidiasis, and liver biopsy was performed. In tissue, there was a mixed non-granulomatous inflammatory infiltrate without neoplasia, fungal, or bacterial elements. After two months of voriconazole, symptoms remained, and new solid lesions were noted in the liver and spleen by CT scan. A second liver biopsy was performed, but the culture remained negative. Antifungal was modified empirically to caspofungin, and a sample of blood was collected to perform mNGS (Karius® test). The test was performed by Karius Laboratory (Karius, Redwood City, CA). The standard mNGS protocol is briefly described. During an acute infection, pathogens leave microbial cell-free DNA (cfDNA) in blood. mNGS workflow starts with nucleic acid extraction of the clinical sample, library preparation, and NGS sequencing. Reads are the basic element produced by DNA sequencing. All DNA of a specimen is sequenced in parallel, resulting in isolation and amplification of both host and pathogen nucleic acid sequenced from the sample. The microbial reads were analyzed to identify the composition and abundance of reads of organisms present after removing host DNA from microbial DNA. Results displayed the concentration of pathogen cfDNA detected per microliter of plasma.[3] The concentration of each microorganism was compared to the same microorganism's concentration(s) reported in the last 1000 specimens tested by Karius. The mNGS test revealed *Candida tropicalis*. Caspofungin was maintained, and three months after, symptoms disappeared, and there was a significant reduction in all lesions.

Case 2

A 46-year-old male with a T acute lymphoid leukemia entered a complete remission following induction therapy and received an unrelated allogeneic stem cell transplant (UNR-SCT). Before the transplant, no important infectious event was documented. UNR-SCT conditioning regimen was cyclophosphamide, thymoglobulin, and total body irradiation. During the SCT-neutropenic phase, he developed febrile neutropenia, with negative blood cultures, and was treated empirically with cefepime and vancomycin with a rapid resolution of fever. The patient recovered from neutropenia after 14 days, but a daily fever started after neutrophil recovery. A tiny filamentous in the mitral valve was observed by echocardiography. Empirical therapy with meropenem and daptomycin was started. Concomitantly, acute graft-versus-host disease (GVHD) was noted in the skin and hepatic markers worsened. A few days after, the patient developed acute respiratory failure with a diffuse pulmonary infiltrate by CT scan. Liposomal amphotericin and trimethoprim-sulfamethoxazole (TMP-SMX) were added to therapy. An endemic coronavirus (HKU1) was identified by multiplex-PCR in bronchoalveolar lavage (BAL). Cultures, Galactomannan, *Pneumocystis jirovecii*-, and tuberculosis-PCR in BAL were negative. At this time, the patient started altering mental status, but the magnetic resonance imaging (MRI) did not reveal any change. A blood sample was collected for mNGS and revealed the presence of *Toxoplasma gondii*, and TMP-SMX therapy was maintained. New transesophageal echocardiography was performed, and the previous mitral findings were no longer observed. Unfortunately, during toxoplasmosis treatment, the patient developed sepsis due to multiresistant *Acinetobacter baumanni* in the following days and died. The patient was *T. gondii*-seropositive (positive IgG and negative IgM) before the transplant.

Case 3

A 34-year-old female with acute lymphoid leukemia in remission received a haploidentical peripheral allogeneic STC (Haplo-SCT) from her brother following conditioning with cyclophosphamide, fludarabine, and total body irradiation. Febrile neutropenia developed during SCT-neutropenic phase, and she was initially treated with empirical cefepime and switched to meropenem and vancomycin due to the persistence of fever despite negative cultures. Neutropenia recovered 16 days after transplantation. On day +18, the patient reported urinary symptoms and gross hematuria. CMV disease was documented (CMV inclusion bodies in bladder tissue and CMV viremia), and ganciclovir was started. Cultures and molecular tests for other viruses (adenovirus, BK, and JC) were negative in urine samples. During ganciclovir therapy, CMV viremia was resolved, but hematuria persisted. Two weeks after, the patient started fever and respiratory symptoms. Nodules, ground-glass infiltrates, and pleural effusion were observed by CT scan. Liposomal amphotericin was started empirically, with a resolution of fever, respiratory symptoms, and radiological improvement. Bronchoalveolar lavage was negative for CMV, galactomannan assay, fungal and bacterial cultures. On D + 60, GVHD was noted in the skin and liver, and corticosteroid was started. Three weeks later, the patient experienced new episodes of fever with moderate to severe respiratory symptoms. A new CT scan showed bilateral infiltrates, centrilobular opacities, and consolidations. Empirical therapies were then restarted, and a sample of blood collected to perform mNGS. Bacterial and fungal cultures, toxoplasmosis, legionella, pneumocystis, and CMV molecular tests were all negative. The mNGS was positive for adenovirus and torque teno virus (TTV). Cidofovir was started, but the patient developed sepsis, renal and respiratory failure, and in a few days, worsened with hemodynamic instability and died.

Discussion

Patients submitted to acute leukemia treatment have considerable risk of infection.[1] Although bacterial and fungi etiologies are most frequent, some diagnostically challenging infections occur and require an intensive investigation and a large diagnostical panel. Intense immunosuppression, dense antimicrobial exposure, and emergency of uncommon agents are important factors related to these diagnostically challenging events. In this report, we summarized three patients with acute leukemia in different phases of therapy with complicated, challenging infections. All of them have had their diagnoses based on mNGS (Karius® test) after an intensive laboratory and imaging investigation with no elucidative diagnosis. The Karius® test is a mNGS of microbial cell-free-DNA able to identify and quantify over 1000 clinically relevant pathogens, including bacteria, fungi, parasites, and DNA viruses. It does not cover RNA viruses nor bacterial resistance profiles. Karius reports the concentration of pathogen cfDNA detected per microliter (MPM). As there is no threshold to distinguish colonization from infection, a committee reviewed all results considering MPM, medical history, physical findings, and conventional laboratory tests to confirm if the result was clinically significant.

The first case presented chronic disseminated candidiasis, a rare presentation of invasive fungal infection in acute leukemia patients nowadays.[4,5] Despite considering invasive fungal infection the probable diagnosis, it was not confirmed by tissue cultures, and the patient experienced radiological progression despite antifungal therapy. Therefore, the mNGS test was useful for confirming the diagnosis and identifying the *Candida* species, which allowed antifungal therapy adjustment and patient's recovery.

In the second case, the mNGS detected an uncommon infection in SCT. Incidence of toxoplasmosis reactivation after SCT is considered rare, depending on the country's prevalence of toxoplasmosis.[6] In Brazil, toxoplasmosis seroprevalence is very high, and TMP-SMX prophylaxis is regularly used in the post-SCT period. Despite the absence of tissue documentation, the mNGS contributed to confirm the etiology leading to adequate therapy. Unfortunately, the patient developed a second infection complication, and death occurred within 30 days after toxoplasmosis diagnosis. Mortality rates of toxoplasmosis in SCT patients are reported as 50% or higher by some studies.[6,7]

In the last case, mNGS detected disseminated adenoviruses and a recently described virus. Adenovirus can cause a devasting disease in SCT patients. Hemorrhagic cystitis, pneumonia, enteritis, and hepatitis are clinical manifestations of invasive adenovirus, especially after high immunosuppression SCT.[8] Torque teno virus (TTV) DNA is commonly found in the plasma of healthy blood donors being considered a non-pathogenic member of the human virome.[9] TTV viremia has been evaluated as a surrogate marker of immune function.[10]

Although mNGS has been applied in several scenarios, some mNGS reports published in leukemia patients showed a contribution considering the broad spectrum of diagnosis possibilities and the consequence of infectious complications in immunocompromised hosts.[11,12]

In this study, mNGS obtained from patients with acute leukemia improved the diagnosis of challenging infection diseases and provided the opportunity for adequate therapy.

Ethics approval

This study was approved by the institutional review borders of Hospital 9 de Julho (reference Number: 30907420.1.0000.5455).

Conflicts of interest

The authors declare no conflicts of interest.

Authors contributions

MG, PVO, and MM were involved in the clinical management of patients. LCP, and AC were involved in laboratorial diagnosis. This manuscript was initially drafted by MG, LCP, and AC and then revised by other authors in the study. All authors read and approved the final manuscript.

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