

Methylobacterium Radiotolerans Bacteremia in Hemodialysis Patients: The New Technique of Identification MALDI-TOF Mass Spectrometry

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ABSTRACT

Central venous catheter (CVC) infections represent one of the most common complications in hemodialysis patients. Understanding the risk factors that induce CVC infection and identifying the microorganisms that cause them, is essential for developing preventive strategies. Therefore, it is important to develop prophylaxis and surveillance protocols, but also to determine adequate diagnostic criteria that include the use of new laboratory tools, useful in identifying a wider range of pathogenic microorganisms. We reported a clinical case of CVC related infection caused by the gram negative *Methylobacterium radiotolerans* in a hemodialysis patient. Matrix-assisted laser desorption/ionization-time of flight is a type of mass spectrometry (MALDI-TOF MS) which utilizes microbial protein mass distribution analysis, generating a signal that is decoded and represented as a peak on a Cartesian reference system. MALDI-TOF MS was fundamental in the identification of the pathogen, which remained previously unidentified using routine laboratory techniques. The use of MALDI-TOF MS has therefore allowed the pathogen's tempestive identification, accurate diagnosis and targeted antibiotic treatment, positively impacting on infection resolution, prognosis and patient survival.

Keywords: *Infections, Hemodialysis, MALDI-TOF MS*

Introduction

Central venous catheter (CVC) related infections represent one of the most common complications in hemodialysis patients. Understanding the risk factors that induce CVC related infection and identifying the microorganisms that cause them, is essential for developing preventive strategies. In fact, CVC infectious complications are associated with an increase in mortality and morbidity. Therefore, it is useful to develop prophylaxis and surveillance protocols but also to identify adequate diagnostic criteria that include the use of new laboratory tools to detect a wider range of pathogenic microorganisms. Currently,

routine laboratory techniques often do not identify the pathogen, resulting in the use of empirical and non-targeted antibiotic therapies, not allowing the rescue of the dialysis intravascular devices (Karkar, 2018).

We hereby present a clinical case in which a new laboratory technique, called Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), which made it possible to identify a pathogen that had not previously been assessed with routine techniques used in microbiology.

Methylobacterium radiotolerans is a Gram-negative bacillus. It is an obligate aerobe belonging to the methylobacteriaceae family. It grows with difficulty on traditional culture media; however, if it does, the growth occurs after 4-5 days incubation at 25°-30°C; colonies will appear small and pink. The best culture terrain for this species is Sabouraud agar, however it can also grow on modified Thayer-Martin, buffered charcoal yeast extract and Middlebrook 7H11 agar. When Gram stained, the microorganisms appear as straight, or slightly curved, pleomorphic, asporogenous, vacuolated bacilli which are resistant to discoloration (Kovaleva *et al.*, 2014; Li *et al.*, 2015).

These microorganisms are resistant to toxic substances, such as detergents and disinfectants, as well as high temperatures and dehydration. They have the capacity to form a biofilm, which enables them to create ecological niches within medical apparatus, intravascular devices and dialysis water treatment systems (Truant *et al.*, 1998).

M. radiotolerans can cause infections in immunocompromised patients, such as those affected by either solid or hematological malignancies, human immunodeficiency virus, chronic kidneys disease, alcoholism and intravascular device carriers (port-a-cath, CVC) (Lai *et al.*, 2011; Li *et al.*, 2015; Wang *et al.*, 2016; Kaneko *et al.*, 2020). Hemodialysis patients present an elevated risk to develop infections due to their immunocompromised status, brought about by neutrophil dysfunction and concomitant uremic toxins accumulation, malnutrition, secondary hyperparathyroidism, altered glucose metabolism, and iron overload. In fact, end-stage renal disease patients are susceptible to infections as uremia is associated with an alteration of the immune system, characterized by immunosuppression and enhanced inflammatory status and oxidative stress (Kato *et al.*, 2008; Noce *et al.*, 2014; Noce *et al.*, 2019).

Additionally, the necessity to use a CVC favors the development of systemic infections. Hemodialysis itself represents an independent risk factor for infections, because of issues regarding inadequacy of treating dialytic water and CVC management by the sanitary personnel. Finally, the interruption of the physiologic cutaneous barrier due to the presence of an intravascular device can favor microbiological colonization, which is greater in these patients compared to those who have a different

type of vascular access (like prosthetic and native arteriovenous fistula, AVF) (Jaber, 2005; McCann and Moore, 2010; Santoro *et al.*, 2014; Fisher *et al.*, 2020).

Hemodialysis CVC long-term infections represent from 48% to 73% of infections and cause 23% hospitalization in HD patients (Sarnak and Jaber, 2000; Allon *et al.*, 2003).

The majority of infections are caused by Gram positive bacteria, prevalently by coagulase negative staphylococci, however in 25 to 40% of cases they are caused by Gram negative ones (de Cal *et al.*, 2009; Eleftheriadis *et al.*, 2011; Golestaneh and Mokrzycki, 2018).

The principal mechanisms which determine the development of a CVC infection start when the bacteria, colonizing the CVC exit-site, migrate through the subcutaneous tunnel and reach the tip of the catheter. Then, there is contamination of the catheter lumen, through hub colonization, infusion of non-sterile substances (rarely) or secondary to systemic bacteremia (Gahlot *et al.*, 2014).

Contamination can also be due to local factors such as sanitary personnel poor hygiene, compromised integrity of the exit-site, and failure to apply CVC care guidelines (Ferrara and Albano, 2018).

Criteria which define a CVC related infection are tenderness by palpation, a positive blood culture, fever and the absence of alternative infection sources.

Treatment of CVC related infections follows a precise diagnostic algorithm which varies according to the presence of complications such as: abscess formation, septic thrombosis, endocarditis, and to the bacterial or fungal species isolated in culture. Complicated infections, independently from the microorganism involved, and infections caused by *Candida* and *Staphylococcus Aureus* must always be treated by removing the CVC and administering targeted antibacterial/antifungal therapy. Non complicated infections caused by other Gram positive or negative microorganisms are generally treated with systemic antibiotic therapy, lock therapy and catheter salvage. Only in resistant cases (presence of fever, persistently positive blood cultures and evidence of septic complications), is CVC removal indicated (Mermel *et al.*, 2009; Tazza *et al.*, 2010; Schmidli *et al.*, 2018).

Case Presentation

A 67 year old man came to the hemodialysis facility in the Fondazione Policlinico Tor Vergata of Rome (Italy) in November 2018, in order to undergo a chronic dialytic treatment three times *per week*. The patient had started hemodialysis in July 2017 at another facility. He was affected by type II diabetes mellitus, diffuse atherosclerosis, dyslipidemia, arterial hypertension and ischemic cardiopathy (previous

myocardial infarction treated by angioplasty with stent insertion), previous cerebral ictus, end-stage renal disease in hemodialysis treatment since July 2017. He presented a clinical history of previous failed vascular access (arteriovenous fistula thrombosis, and suspected CVC related infection with fever persistency and sterile blood culture). During a routine physical examination, he referred post dialysis serotonin fever which lasted for at least a year. Laboratory data allowed the exclusion of a hematological neoplasia, a suspect which was initially proposed because of an oligoclonal hypergammaglobulinemia in the absence of hematocrit abnormalities and light chains. A slight increase in inflammation indexes (C-reactive protein, CRP and procalcitonin, PCT) was observed. On the basis of the anamnesis, clinical symptomatology, and laboratory data, a set of blood cultures was sampled before and after dialytic treatment from the peripheral veins and from the CVC. The post-HD blood culture taken in date 27/11/2018 resulted positive for the growth of *M. radiotolerans* colonies (Table 1). The microorganism was identified thanks to MALDI-TOF MS, and it resulted sensitive to all tested antibiotics. Considering the personal history of thrombophilia, previous failed vascular accesses, and the presence, of the superior bilateral superficial venous reticulum suggestive of central venous thrombosis, an attempt to salvage the vascular access was performed by sterilizing the CVC using brushing and lock-therapy with Ceftriaxone (antibiotic) plus 100.000 UI of urokinasis. Moreover, he was administered an antibiotic therapy with 2 gr/day endovenous Ceftriaxone for 10 days.

Table 1. Blood culture sampled from CVC.

MICROBIOLOGY AND VIROLOGY			
BACTERIOLOGY			
Bacteria microscopic research (whole blood):		Gram-Negative Bacilli	
Material	Whole blood		
Isolated			
POSITIVE OF 01/12/18 TD 102.7			
R: resistant - I: susceptible, increased exposure - S: susceptible			
Antibiogram interpreted according to the EUCAST criteria.			
Medical reports of 03/12/2018 8.05			
Aerobic bacteria blood culture (CVC):			
Material	blood by CVC		
Isolated	<i>Methylobacterium radiotolerans</i>		
Ampicillin	0.5	S	
Ampicillin/sulbactam	0.75	S	
Ceftriaxone	0.75	S	
Levofloxacin	0.38	S	
Meropenem	0.047	S	
Penicillin G	0.64	S	
Piperacillin/tazobactam	0.19	S	
POSITIVE OF 01/12/18 TD 102.7			
R: resistant - I: susceptible, increased exposure - S: susceptible			
Antibiogram interpreted according to the EUCAST criteria.			

After 10 days, the resolution of the clinical picture was observed. A series of negative blood cultures were sampled, and there was a progressive improvement and normalization of inflammatory indexes, hypergammaglobulinemia resolution and disappearance of fever.

Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry

There are three techniques which are used to perform bacterial identification and phenotypical characterization, namely the biochemical method, the molecular method and the new spectrometry technique MALDI-TOF MS (Nagorny *et al.*, 2019). MALDI-TOF MS has high accuracy and rapidity, allowing the identification of different microorganism directly from the sample, which is difficult to perform with other techniques. The colony is taken from a culture plate and placed on the MALDI-TOF MS “target” plate. Adding formic acid can improve the quality of generated mass spectra, which is particularly useful for some types of microorganisms, such as yeasts. After desiccation, the “target” plate is inserted in the ionization chamber. The MALDI matrix (for example α -Cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile and 2,5% trifluoroacetic acid) assists in the desorption and ionization of the microbial analytes brought about by the energy of the laser. The matrix isolates the analyzed molecules and protects them from the fragmentation that could be caused by the laser. Once hit by the laser, microbial species and matrix molecules are de-absorbed, with most of the energy absorbed by the matrix, which converts to an ionized state. The interaction among the photons from the laser and matrix molecules caused by uptake of energy from the beam triggers a sublimation of the matrix into a gas phase followed by the ionization of the specimen. Soft ionization of proteins is critical for bacterial identification methods, as it allows the analysis of large biomolecules, including ribosomal proteins. Once ionized, proteins are analyzed by a component of the mass spectrometer called the mass analyzer to reveal characteristic information about the composition of the sample in the context of a spectrum of mass-to-charge (m/z) ratios. This process generates a spectrum which is compared to a database of defined reference spectra, which leads to microbial identification.

In particular, thanks to random collisions during the gaseous phase, the charge is transferred from the matrix to the microbial molecules. The ionized molecules undergo acceleration based on their mass/charge ratio in a vacuum tube TOF mass analyzer. The ions travel towards an ion detector, the smaller analytes reach the detector first, followed by progressively larger ones. A mass spectrum is generated, which represents the number of ions in given mass impacting the ion detector in a given time. The mass spectrum is then compared to a database of spectra by using a software, which will then allow the identification of the microorganism.

Particularly abundant microbial proteins such as ribosomes, majorly contribute to the mass

spectrum generation, even if the specific proteins have not been identified and only their mass and abundance have been profiled.

Generally, mass spectra are unique for each type of organism, with specific peaks for genera, species and strains. The isolated mass spectrum is compared with a reference spectra database (or deconvolution spectra) in order to determine the relationship between the examined sample and the existing spectra. The more closely correlated microorganisms are identified with a value which corresponds to the level of confidence in the identification. Depending on how high this value is, the organism's family, genera or species can be characterized. A variety of algorithms are used to perform database comparisons. Finally, in order to expand the database variety, there are some specialized facilities which create entire mass spectra libraries and give the possibility to the most modern MALDI-TOF MS instrumentations to create their own personal library and to share it online with other sanitary professionals, hospital companies, and research centers, thus notably expanding the capacity to identify microbial species.

Discussion

MALDI-TOF MS has become the election method to identify the major aerobic and anaerobic bacteria, substituting 16S rRNA gene sequencing and others traditional biochemical methods of pathogens identification. Many clinical microbiology labs did not have instruments adept to the identification of anaerobic bacteria, therefore, this can be done with MALDI-TOF MS. This method allows an enhancement in routine identification of microorganisms, probably increasing the information in the interpretation of the clinical significance and the expected sensibility of the anaerobes. The whole process is completely automated, quick and cheap; it allows the rapid individuation the pathogens and promptly initiate targeted therapy resulting in improved diagnostic accuracy, increase in survival, and minor sanitary costs especially regarding hospitalization necessity and hospital stay days (Patel, 2015; Cordovana *et al.*, 2019). The possibility to archive previously identified species, will allow swifter successive identification of these species. Thus, allowing more tempestive and targeted antibiotic treatment. Therefore, such methodology offers the opportunity of lowering sanitary costs associated with the management of patients with infections caused by pathogens which are hard to identify with routine laboratory techniques.

Author's Contributions

Conceptualization: A.N. and N.D.D.; clinical assistance: E.O., M.F. and P.B.; writing—original draft preparation: G.M., C.D.A., G.G. and M.D.L.; Review and editing: G.W.J. and C.F.

All authors provided substantial intellectual input to the work and approved the manuscript final version.

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