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BMJ Open Diagnostic performance of the DISQVER metagenomic sequencing tool for the identification of pathogens in febrile neutropenic patients: the ADNEMIA trial

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ABSTRACT

Introduction While intensive protocols in onco-haematology have improved survival rates for patients with haematological malignancies, they have also resulted in an increased incidence of infection associated with therapy-induced immunosuppression (including chemotherapy-induced febrile neutropenia; FN). The occurrence of FN, associated with high morbidity and mortality, necessitates broad-spectrum antibiotic therapy, occasioning delayed chemotherapy and resulting in a loss of opportunity for the patient. Considering that without an identified pathogen, a 10% mortality rate can ensue, documentation is essential to the optimisation of antibiotic therapy. However, blood culture (the reference test) is limited for several reasons: such as fastidious culture, antibiotic treatment prior to sampling or insufficient sample volume. Sequencing technologies have led to the development of diagnostic approaches based on the detection of circulating DNA in blood. This study will aim to assess the clinical utility of metagenomic next-generation sequencing (mNGS)-DISQVER technology in detecting pathogenic microorganisms from blood samples of patients undergoing high-risk FN treatment.

Methods and analysis This nationwide, prospective, multicentre, interventional, proof-of-concept clinical trial will enrol 200 patients. Will include patients ≥18 years old, treated for malignancy, at high risk of FN (Multinational Association for Supportive Care in Cancer score ≤21) with an expected duration of neutropenia ≥7 days. Patients who received antibiotic treatment within 24 hours prior to enrolment, have previously participated and/or have enhanced protection will be excluded. The primary outcome will be determined by considering the microorganisms responsible for this FN, weighted by the assessment of an adjudication committee. Secondary outcomes will evaluate patient management depending on the arm. The second secondary outcome will be determined by the duration of conventional assessment, frequency of microorganisms detected during routine care and percentage distribution of theoretical adjustments made to anti-infective treatment based on microorganisms diagnosed using the mNGS-DISQVER tool as compared with conventional practices. Identifying the pathogens responsible for high-risk FN from a blood sample, using an unbiased technique, can provide microbiological

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This study focused on febrile neutropenia as these patients have a critical need for broad and rapid technologies, especially in the sepsis situation.
- ⇒ This study aims to evaluate metagenomic next-generation sequencing technologies in real patients.
- ⇒ In order to assess the clinical benefit of this technique, an adjudication committee has been constituted.
- ⇒ A nationwide randomised interventional trial is required to assess the direct benefit of using this technology which is currently lacking in the present protocol.
- ⇒ In order to assess the value of using this technology, it is necessary to conduct an economic evaluation which is absent from the existing protocol.

documentation and may even reveal unexpected microorganisms in these profoundly immunocompromised patients.

Ethics and dissemination The protocol received approval from the Comité de Protection des Personnes Sud-Méditerranée II. All participants will provide informed consent before participation. The trial has been registered on ClinicalTrials.gov (identifier NCT06075888). The results of the main trial and each of the secondary endpoints will be submitted for publication in a peer-reviewed journal.

Trial registration number ClinicalTrials.gov
[NCT06075888](https://clinicaltrials.gov/ct2/show/study/NCT06075888).

INTRODUCTION

The development of targeted therapies and intensive protocols in onco-haematology has improved the survival of patients with haematological malignancies.¹ However, the increased number of patients treated and their longer life expectancy have led to increased incidence of infectious complications secondary to the immunosuppression induced by these therapies.



Chemotherapy-induced febrile neutropenia (FN) is a frequent and serious complication that occurs in approximately 10–15% of patients treated for solid tumours and in up to 100% of patients treated for haematological malignancies, particularly after bone marrow transplantation.^{2,3} FN is defined as a combination of a neutrophil count $<500 \times 10^9/L$ or $<1000 \times 10^9/L$ if $<500 \times 10^9/L$ was expected within 48 hours and fever $>38.5^\circ\text{C}$ on a single occasion or $>38^\circ\text{C}$ for more than 1 hour.

FN is associated with high morbidity and mortality (25–30% of serious complications and 10% of mortality). Its occurrence entails a need for broad-spectrum antibiotic therapy, delayed chemotherapy and loss of opportunity for the patient. In the USA, each episode of FN requiring hospitalisation is associated with an estimated economic health impact of US\$19 110.⁴ A recent French study estimated the clinical and economic impact of hospitalisation for FN, in terms of the number of patients hospitalised, number of stays, in-hospital mortality and the average cost per stay, at €5333 for haemopathies.⁵

In these situations, infections are the primary cause of illness and death, accounting for more than 50% of fatalities.^{6–8} A significant proportion of FN cases, caused by microbial infections, can progress to septic shock if empiric broad-spectrum antibiotic therapy is not administered rapidly.⁹ Furthermore, microbiological documentation remains essential for optimised antibiotic therapy, with blood culture as the reference test. However, this test is limited for several reasons: fastidious and prolonged growth of bacteria, antibiotic treatment prior to sampling or insufficient sample volume. Despite the recommendation that a minimum of three or four pairs of blood cultures be obtained, most FN cases are not microbiologically documented, making it impossible to adapt empiric to broad-spectrum antibiotic therapy (ref). Apart from the particularities of managing such therapies, such treatments have very detrimental consequences on the various microbiota of the individuals concerned, including resistome expansion. Another limitation is the time lapse required to obtain results: 6–24 hours are required to obtain a positive blood culture, plus another 18–24 hours for bacteria to grow on solid media and yet another 18–24 hours for antibiotic susceptibility testing.

The absence of an identified pathogen in management of the most severe patients is associated with a poor prognosis, with a mortality rate of 10%.¹⁰ In this context, the implementation and timeliness of diagnostic strategies are important factors in improving the prognosis of these patients. In recent years, the development of high-throughput sequencing technologies has led to the development of new diagnostic approaches based on the detection of circulating DNA (lcDNA, eg, used in antenatal screening for trisomy 21). Although only recently democratised, the concept of microbial cDNA is currently well-established in the field of infectious diseases.^{11,12} This technology, based on a blood sample obtained by simple venipuncture, could offer patients with FN an improved

diagnosis, particularly in the event of negative blood cultures, and adjustment in their management.^{13–16}

DISQVER is a digital analytical solution developed by Noscendo for the rapid, hypothesis-free identification of microorganisms in a patient's blood sample from free circulating DNA. The test is based on metagenomic next-generation sequencing (mNGS) to identify, without a priori assumptions, the genomic sequences of microorganisms (bacteria, DNA viruses, fungi and parasites) potentially present in a patient's plasma sample. The identified genomic sequences will be subsequently compared with the Noscendo proprietary database of more than 16 000 microbial genome sequences, including 1500 pathogens.

The objective of this research will be to evaluate the clinical benefit of using mNGS-DISQVER technology for the identification of pathogenic microorganisms in blood samples obtained from patients undergoing high-risk FN treatment, in comparison with the standard clinical management approach.

METHODS AND ANALYSIS

Selection criteria

Patients meeting the following criteria will be eligible for inclusion: (1) aged 18 years or older; (2) receiving treatment for a solid tumour or haematological malignancy; and (3) presenting with high-risk FN, defined as a Multinational Association for Supportive Care in Cancer score of 21 or lower, with expected neutropenia duration of 7 days or longer. Neutropenia will be defined as an absolute neutrophil count of $1500/\text{mm}^3$ or less and fever as a temperature of 38°C or higher. Patients having provided informed consent to participate in the study and covered by a social security scheme or a third party will be eligible (legal considerations).

The exclusion criteria will be as follows: (1) patients receiving antibiotic therapy within the last 24 hours prior to enrolment, except for prophylactic use of trimethoprim-sulfamethoxazole and penicillin G; (2) patients who had previously participated in this study (except when first sample remains technically non-conclusive); and (3) patients with enhanced protection (ie, minors, individuals deprived of their freedom by a judiciary or administrative decision, those residing in a medical or social care institution, adults under legal guardianship; and pregnant or breastfeeding females of childbearing age considering that menopause must be documented who declined or lacked access to an effective contraception method, that is, hormonal/mechanical—oral, injectable, transdermal, implantable, intrauterine device or surgical—tubal ligation, hysterectomy, complete oophorectomy, during the study period).

The recruitment process began in January 2024 and involved five university hospitals (Poitiers, Tours, Brest, Angers and Limoges). Ethical approval for the study was obtained from the Comité de Protection des Personnes (CPP) Sud-Méditerranée II (accession number

2023-A01438-37). It is anticipated that the study will be concluded by July 2025.

Outcome measures

The primary outcome will be determined by considering the microorganisms responsible for this FN as part of the patient management plan. This determination will be made by the adjudication committee which will consider the potential presence and characteristics of identified microorganisms through conventional diagnostic procedures or the mNGS-DISQVER tool.

The index test will consist in the mNGS-DISQVER analysis. Briefly, blood samples will be collected in Streck Blood Collection tubes and shipped at room temperature by medical logistics to the laboratories of Noscendo GmbH in Reutlingen, Germany. Plasma separation and aliquoting, nucleic acid isolation, quality control and library preparation will be performed as described.^{13 14}

Adequate positive and negative controls will accompany all laboratory and sequencing procedures. Raw sequencing data will be subjected to various quality controls including Phred score filtering, adapter trimming, complexity filtering and K-mer-based contamination screening, as previously described. All data generated will be analysed using Noscendo DISQVER platform. The DISQVER platform comprises a curated microbial genome reference database of over 16 000 microbial species covering more than 1500 pathogens and can detect bacteria, DNA viruses, fungi and parasites, while differentiating contaminants and commensals from infectious agents. The mNGS report will typically be available within 48 hours of sending the sample to the coordinator laboratory.

In order to be limited to the diagnostic procedures that are invariably considered in the context of FN, the conventional work-up will include, as described in the protocol section, blood culture, urine culture, Epstein-Barr virus (EBV) and/or Cytomegalovirus (CMV) PCR, *Aspergillus* serology and antigen and β -D-glucan determination. Despite suggestions in some published literature, phenotype classification will not be a necessary component of this management arm.¹⁷ This is due to the fact that the referral laboratories in the various investigating centres possess disparate skill sets which consequently result in varying levels of capacity for delivering results including analyses that may or may not be accessible. The objective of this management arm is to represent, to the greatest extent possible, the most straightforward approach to the management of the investigating laboratories.

Due to the clinical and biological nature of the outcome, an adjudication committee composed of 10 hospital practitioners (microbiologists, specialists in infectiology and haematologists), who will not be investigators of the study, will be constituted. A team comprising a clinician and a biologist will be established to determine whether the microorganisms responsible for this FN should be taken into account. The pair will have access to all data, with the exception of mNGS-DISQVER, in order to make decisions regarding management in the conventional

arm. In addition, they will have access to all data, with the exception of blood and urine cultures, PCR, antigens and serologies, in order to make decisions regarding management in the mNGS-DISQVER arm. A consensus will be achieved or a third practitioner will expertise the clinical case.

The secondary outcome will evaluate patient management methods (escalation, maintenance, de-escalation or discontinuation of anti-infective treatment) using the mNGS-DISQVER tool compared with the conventional work-up. The second secondary outcome will be determined by the duration of conventional assessment, frequency of microorganisms detected during routine care (independent of the adjudication committee) and percentage distribution of theoretical adjustments made to anti-infective treatment based on microorganisms diagnosed using the mNGS-DISQVER tool in comparison to conventional practices.

Protocol

The study will enrol 200 patients. This is an interventional, nationwide, prospective, multicentre, proof-of-concept clinical study with minimal risks and limitations designed to evaluate the benefit of using a European Community (CE-) marked medical diagnostic device when used as intended, with results that will not influence patient management decisions or be used to guide care or entail additional cumbersome/invasive procedures (table 1).

As part of routine care, the patients will be interviewed about their demographics (age, sex), medical history, current treatments or those taken in the last 24 hours, duration of neutropenia before the infectious episode and history of high-risk FN. Patients will undergo a clinical examination to measure temperature, heart rate, blood pressure, arterial oxygen saturation and Glasgow score.

The following samples will be taken (figure 1): blood culture (5–10 mL of blood taken in two to three pairs, culture according to the national recommendations of "Referenciel en Microbiologie Clinique" (REMIC) and "Comité de l'Antibiogramme de la Société Française de Microbiologie" (CA-SFM), depending on the machines available in each of the study centres), urine culture, EBV/CMV PCR (5 mL of blood on EDTA), *Aspergillus* serology and antigen and β -D-glucan determination (5 mL of serum, analysis on automated equipment available in each centre) and haemogram (5 mL of blood, analysis on automated equipment available in each centre) samples.

As part of the research, 10 mL of blood will be collected in Streck tubes (with preservatives and anonymisation using a QR code). These tubes will be stored at 4°C until they are sent once a week by courier to the Noscendo laboratory (Duisburg, Germany).

Analysis

On receipt, the samples will be processed within 48 hours for metagenomic analysis using an accredited method as part of the mNGS-DISQVER diagnostic kit. The

Table 1 Timeframe of the ADNEMIA study

	Initial visit	End of the hospitalisation (or Day 30 maximum)
Selection criteria	X	
Information about the patient	X	
Consent collection	X	
Clinical examination	X	
Demographic information and history of the disease	X	
Duration before an infectious episode of high-risk FN	X	
Anamnesis of high-risk NF	X	
Treatment for high-risk NF		X
Conventional sampling	X	
mNGS-DISQVER sampling	X	
Clinical status		X

mNGS, metagenomic next-generation sequencing.

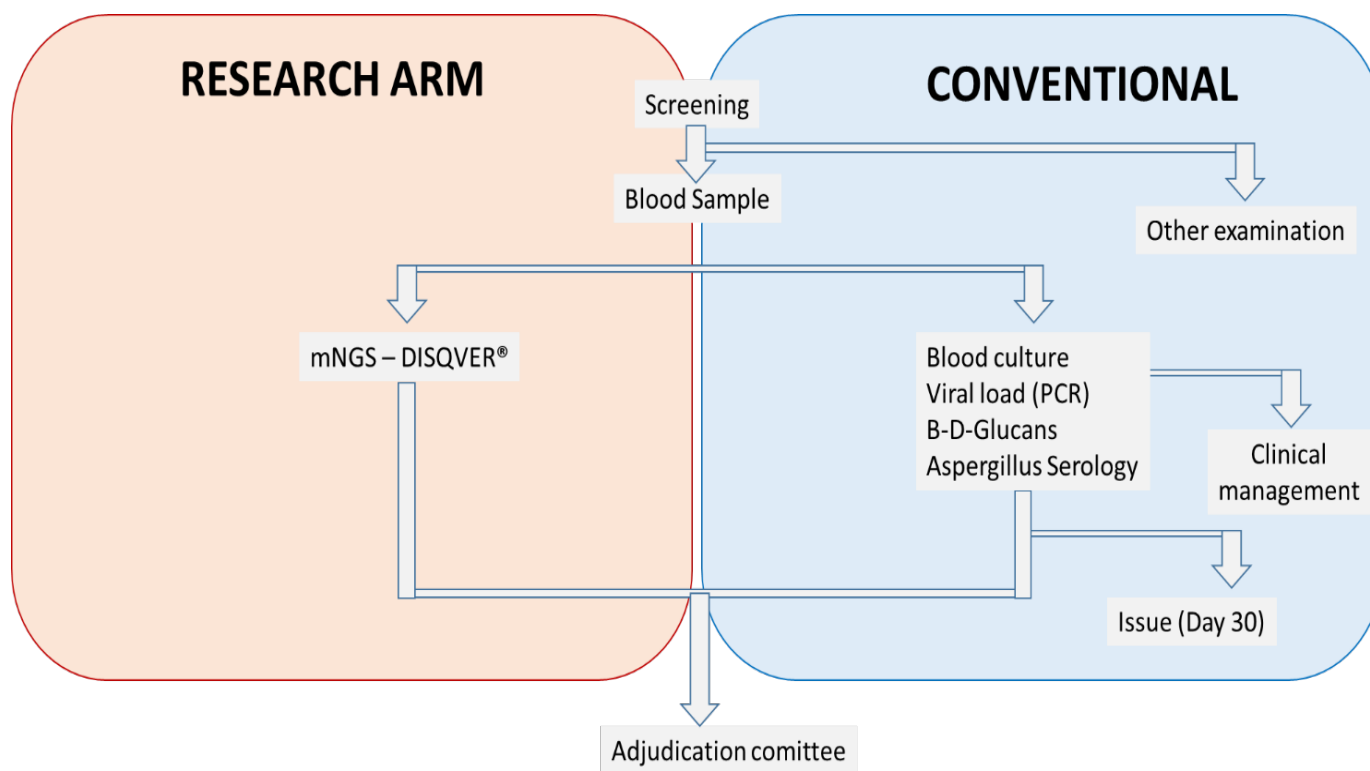
technician performing the mNGS-DISQVER test will be unaware of the results of routine microbiological testing.

A standardised, pseudonymised metagenomic sequencing report including sample identification, technical results (sequencing quality) and biological results (microorganisms found and putative interpretation of the pathogenic microorganism(s) identified) will be sent to an independent microbiologist so that he can enter the list of microorganisms identified by the mNGS-DISQVER technique into the electronic Clinical Recording File (e-CRF) independently of the results of the conventional

work-up. These results will not be communicated to the patient or doctors having managed the patient.

Adjudication committee

An adjudication committee of non-investigator microbiologists, infectious disease specialists or haematologists from different participating centres will discuss at the end of the study to categorise the episodes of high-risk infectious FN and whether the results of the mNGS-DISQVER method would have influenced the treatment of the patient received.

**Figure 1** ADNEMIA study implementation diagram. mNGS, metagenomic next-generation sequencing.

In short, interpretation of the conventional assessment and that provided by the mNGS-DISQVER tool will be blinded, that is, the members of the committee will not know (1) the identity of the patient; (2) the treatment(s) given to the patient by the centre following the results of the conventional assessment; (3) the results of the conventional assessment when they assess the results of the mNGS-DISQVER tool; and (4) the results of the mNGS-DISQVER tool when adjudicating the results of the conventional assessment.

Power calculation

In the absence of data from the literature at the outset of the study, it was challenging to ascertain the number of patients required to assess the benefit of using the mNGS-DISQVER tool.

According to the capacity of centres to include 40–50% of FN cases of infectious origin during the inclusion period, 200 episodes of high-risk FN could be analysed. It is reasonable to assume that each of the enrolling centres will be able to enrol 40 patients during the inclusion period for this exploratory study.

Statistical analysis

For this interventional study, the description of patients at enrolment will consist of estimating percentages for qualitative variables and means and SD for quantitative variables.

The diagnostic abilities of the new mNGS-DISQVER test will be evaluated based on its sensitivity and specificity, using the conventional work-up classification as the benchmark. Additionally, the Youden index, as well as the positive and negative predictive values of the new mNGS-DISQVER test (where the conventional work-up is the reference) and the proportion of concordant and discordant pairs between the new test and conventional work-up, will be assessed.

The differences between the percentages of patient management modalities (escalation, stability, de-escalation or cessation of anti-infective treatment) using the mNGS-DISQVER tool versus the conventional reference assessment will be estimated. A Cochran's Q test (comparison of two random variables with more than three modalities for paired data) will be performed (5% alpha risk).

Finally, a description of the management observed in real life will be produced: the observed average time required for conventional assessment, the prevalence of microorganisms diagnosed in routine care (independent of the adjudication committee) and the percentage of anti-infective adjustments according to microorganisms diagnosed in routine care.

All of these indicators will be associated with their 95% CIs estimated by non-parametric bootstrapping (some numbers are expected to be too small to assume asymptotic hypotheses). Analyses will be performed with an alpha risk of 5%.

It should be noted that the study will be conducted on a complete case basis. To assess any selection bias, the

patients analysed will be compared with those excluded for missing data.

Patient and public involvement

None.

DISCUSSION

Identification from a blood sample of pathogens responsible for high-risk FN in immunocompromised subjects without any a priori hypothesis could allow microbiological documentation, even for unexpected microorganisms, in cases where conventional techniques may fail. The Ninth European Conference on Infections in Leukaemia clearly stated that antibiotic de-escalation is only possible if microbiological documentation is available.¹⁸ Antibiotic de-escalation, with good microbiological documentation, is not associated with a more frequent recurrence of fever and/or bacteremia.¹⁹ In addition, clinical deterioration leading to intensive care treatment remains very rare.^{20–23} Using a high-performance, unbiased and rapid approach such as the one tested in the protocol will enable us to reduce the microbiological impact more quickly and with greater safety.^{15 24–27}

It is important to consider that this approach for diagnosing bacteria, viruses and fungi has limitations, despite its potential benefits in terms of microorganism variability. Currently, the democratisation of this technique is still limited. Although the recent pandemic has enabled many laboratories to be equipped with high-speed sequencing technologies, it is important to note that, as of now, this approach is limited to reference laboratories such as university hospitals. However, the cost efficiency of a broad syndromic approach offsets this limitation, as demonstrated by syndromic PCR.²⁸ Additionally, the routine use of bioinformatic analyses remains a significant challenge in medical biology. To address this issue, an all-in-one interpretation system can be used to optimise interpretation, making it accessible to laboratories that are not specialised in bioinformatics. Finally, it is important to consider the applicability of the identified elements in optimising the appropriate use of antibiotics even if the current version does not provide information on antibiotic resistance profiles (even in genomic form).

Adaptation to antimicrobial or immunosuppressive treatment according to the aetiological diagnosis of the infection could optimise patient management. Finally, in addition to individual benefits, the informed choice of targeted therapeutic modification (escalation, de-escalation or discontinuation) is part of the effort to combat antimicrobial resistance.

ETHICS AND DISSEMINATION

The present study complies with all the ethical requirements and with the protection of the participant subjects as it complies with the legal ethical French regulation and is conducted according to the Declaration of Helsinki in



its last revision. Since it is considered that the patients' participation carries minimal risk., this risk is considered as similar to the one the patient would have throughout usual clinical practice without participating in the study. The protocol received approval from the CPP Sud-Méditerranée II before the inclusion of the patients (National number: 2023-A01438-37) dated 19 September 2023. All participants will provide informed consent before participation. The study protocol was registered on ClinicalTrials.gov with trial registration number NCT06075888. The results of the main trial and each of the secondary endpoints will be submitted for publication in a peer-reviewed journal irrespective of the outcomes.

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Contributors MP: Conceptualisation; Methodology; Software; Validation; Formal analysis; Investigation; Resources; Data curation; Visualisation; Supervision; Writing—original draft; Writing—Review and Editing; Project administration; Funding acquisition. CB: Conceptualisation; Writing—original draft; Writing—Review and Editing; Project administration; Funding acquisition. ADNEMIA Study Group: Writing—original draft; Writing—Review and Editing; MP is responsible for the overall content as guarantor.

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REFERENCES

- 1 Sant M, Minicozzi P, Mounier M, *et al*. Survival for haematological malignancies in Europe between 1997 and 2008 by region and age: results of EURO-CARE-5, a population-based study. *Lancet Oncol* 2014;15:931–42.
- 2 Slobbe L, Waal L van der, Jongman LR, *et al*. Three-day treatment with imipenem for unexplained fever during prolonged neutropaenia

- in haematology patients receiving fluoroquinolone and fluconazole prophylaxis: A prospective observational safety study. *Eur J Cancer* 2009;45:2810–7.
- 3 Buchheidt D, Böhme A, Cornely OA, *et al*. Diagnosis and treatment of documented infections in neutropenic patients—recommendations of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). *Ann Hematol* 2003;82 Suppl 2:S127–32.
- 4 Kuderer NM, Dale DC, Crawford J, *et al*. Mortality, morbidity, and cost associated with febrile neutropenia in adult cancer patients. *Cancer* 2006;106:2258–66.
- 5 Freyer G, Thomé B, Lehmann E, *et al*. Hospitalisations pour neutropénie fébrile chimio-induite en France en 2018: impact clinique et caractéristiques des patients à partir des données de la base PMSI. *Revue d'Épidémiologie et de Santé Publique* 2021;69:S90.
- 6 Hersh EM, Bodey GP, Nies BA. Causes of death in acute leukemia: A ten-year study of 414 patients from 1954–1963. *JAMA* 1965;193:105–9.
- 7 O'Connor D, Bate J, Wade R, *et al*. Infection-related mortality in children with acute lymphoblastic leukemia: an analysis of infectious deaths on UKALL2003. *Blood* 2014;124:1056–61.
- 8 de Lima MC, da Silva DB, Freund APF, *et al*. Acute Myeloid Leukemia: analysis of epidemiological profile and survival rate. *J Pediatr (Rio J)* 2016;92:283–9.
- 9 Kumar A, Roberts D, Wood KE, *et al*. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006;34:1589–96.
- 10 Azoulay E, Pickkers P, Soares M, *et al*. Acute hypoxemic respiratory failure in immunocompromised patients: the Efraim multinational prospective cohort study. *Intensive Care Med* 2017;43:1808–19.
- 11 Han D, Li R, Shi J, *et al*. Liquid biopsy for infectious diseases: a focus on microbial cell-free DNA sequencing. *Theranostics* 2020;10:5501–13.
- 12 Eichenberger EM, de Vries CR, Ruffin F, *et al*. Microbial Cell-Free DNA Identifies Etiology of Bloodstream Infections, Persists Longer Than Conventional Blood Cultures, and Its Duration of Detection Is Associated With Metastatic Infection in Patients With Staphylococcus aureus and Gram-Negative Bacteremia. *Clin Infect Dis* 2022;74:2020–7.
- 13 Grumaz S, Grumaz C, Vainshtein Y, *et al*. Enhanced Performance of Next-Generation Sequencing Diagnostics Compared With Standard of Care Microbiological Diagnostics in Patients Suffering From Septic Shock. *Crit Care Med* 2019;47:e394–402.
- 14 Grumaz C, Hoffmann A, Vainshtein Y, *et al*. Rapid Next-Generation Sequencing-Based Diagnostics of Bacteremia in Septic Patients. *J Mol Diagn* 2020;22:405–18.
- 15 Schulz E, Grumaz S, Hatzl S, *et al*. Pathogen Detection by Metagenomic Next-Generation Sequencing During Neutropenic Fever in Patients With Hematological Malignancies. *Open Forum Infect Dis* 2022;9:ofac393.
- 16 Hong D, Wang P, Zhang J, *et al*. Plasma metagenomic next-generation sequencing of microbial cell-free DNA detects pathogens in patients with suspected infected pancreatic necrosis. *BMC Infect Dis* 2022;22:675.
- 17 Nijman RG, Oostenbrink R, Moll HA, *et al*. A Novel Framework for Phenotyping Children With Suspected or Confirmed Infection for Future Biomarker Studies. *Front Pediatr* 2021;9:688272.
- 18 Maschmeyer G, Bullinger L, Garcia-Vidal C, *et al*. Infectious complications of targeted drugs and biotherapies in acute leukemia. Clinical practice guidelines by the European Conference on Infections in Leukemia (ECIL), a joint venture of the European Group for Blood and Marrow Transplantation (EBMT), the European Organization for Research and Treatment of Cancer (EORTC), the International Immunocompromised Host Society (IHS) and the European Leukemia Net (ELN). *Leukemia* 2022;36:1215–26.
- 19 Groll AH, Castagnola E, Cesaro S, *et al*. Fourth European Conference on Infections in Leukemia (ECIL-4): guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. *Lancet Oncol* 2014;15:e327–40.
- 20 Le Clech L, Talarmin J-P, Couturier M-A, *et al*. Early discontinuation of empirical antibacterial therapy in febrile neutropenia: the ANTIBIOSTOP study. *Infect Dis (Lond)* 2018;50:539–49.
- 21 Snyder M, Pasikhova Y, Baluch A. Early Antimicrobial De-escalation and Stewardship in Adult Hematopoietic Stem Cell Transplantation Recipients: Retrospective Review. *Open Forum Infect Dis* 2017;4:ofx226.
- 22 Petteys MM, Kachur E, Pilling KE, *et al*. Antimicrobial de-escalation in adult hematopoietic cell transplantation recipients with febrile neutropenia of unknown origin. *J Oncol Pharm Pract* 2020;26:632–40.

- 23 Paret R, Le Bourgeois A, Guillermin G, *et al.* Safety and risk of febrile recurrence after early antibiotic discontinuation in high-risk neutropenic patients with haematological malignancies: a multicentre observational study. *J Antimicrob Chemother* 2022;77:2546–56.
- 24 Kalbitz S, Ermisch J, Kellner N, *et al.* Metagenomic next-generation sequencing as a diagnostic tool in the clinical routine of an infectious diseases department: a retrospective cohort study. *Infection* 2024;52:1595–600.
- 25 Balks J, Grumaz S, Mazzitelli S, *et al.* Microbial cell-free DNA-sequencing as an addition to conventional diagnostics in neonatal sepsis. *Pediatr Res* 2024.
- 26 Leitl CJ, Stoll SE, Wetsch WA, *et al.* Next-Generation Sequencing in Critically Ill COVID-19 Patients with Suspected Bloodstream Infections: A Retrospective Cohort Study. *J Clin Med* 2023;12:1466.
- 27 Overbeek R, Leitl CJ, Stoll SE, *et al.* The Value of Next-Generation Sequencing in Diagnosis and Therapy of Critically Ill Patients with Suspected Bloodstream Infections: A Retrospective Cohort Study. *J Clin Med* 2024;13:306.
- 28 Yang M, Tao C. Diagnostic efficiency of the FilmArray blood culture identification (BCID) panel: a systematic review and meta-analysis. *J Med Microbiol* 2023;72.