Pathogen Detection by Metagenomic Next-Generation Sequencing During Neutropenic Fever in Patients With Hematological Malignancies

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Background. Febrile neutropenia (FN) after chemotherapy is a major cause of morbidity during cancer treatment. The performance of metagenomic next-generation sequencing (mNGS) of circulating cell-free deoxyribonucleic acid from plasma may be superior to blood culture (BC) diagnostics for identification of causative pathogens. The aim of this study was to validate mNGS (DISQVER test) for the detection of pathogens in hematologic patients with FN.

Methods. We collected paired whole blood specimens from central venous catheter and peripheral vein during FN for BC and mNGS testing. We repeated paired sampling at the earliest after 3 days of fever, which was defined as 1 FN episode. All clinical data were retrospectively reviewed by an infectious disease expert panel. We calculated percent positive agreement (PPA), percent negative agreement (PNA), percent overall agreement (POA), and sensitivity and specificity.

Results. We analyzed a total of 98 unselected FN episodes in 61 patients who developed predominantly FN after conditioning therapy for allogeneic ($n = 22$) or autologous ($n = 21$) hematopoietic stem cell transplantation. Success rate of mNGS was 99% (97 of 98). Positivity rate of mNGS was 43% (42 of 97) overall and 32% (31 of 97) excluding viruses compared to 14% (14 of 98) in BC. The PPA, PNA, and POA between mNGS and BC were 84.6% (95% confidence interval [CI], 54.6% to 98.1%), 63.1% (95% CI, 51.9% to 73.4%), and 66% (95% CI, 55.7% to 75.3%), respectively. Sensitivity for bacteria or fungi was 40% (95% CI, 28.0% to 52.9%) and 18.5% (95% CI, 9.9% to 30.0%), respectively.

Conclusions. Pathogen detection by mNGS (DISQVER) during unselected FN episodes shows 2-fold higher sensitivity and a broader pathogen spectrum than BC.

Keywords. febrile neutropenia; blood culture; infection; metagenomic; stem cell transplantation.

Febrile neutropenia (FN) after chemotherapy is a major cause of treatment morbidity and mortality in patients with cancer requiring urgent intervention [1, 2]. A significant portion of FN cases is caused by bloodstream infections (BSIs) that can evolve to sepsis if empiric broad-spectrum antibiotic therapy is not initiated promptly [3]. Sepsis represents one of the main causes of death, with mortality rates of up to 30% to 50% in intensive care units [4].

In FN, the causative microbiological pathogen may include both common and uncommon pathogens ranging from bacteria to viruses and fungi. The timely diagnosis of the infection and the identification of the causative pathogen is of major importance to reduce morbidity and mortality and to improve the outcome of FN patients [5]. Rapid detection of pathogens would allow for early targeted antimicrobial therapy and may lead to less drug-related side effects, less antibiotic resistance, reduced medical expenses, and increased survival rates [6, 7]. However, despite extensive medical research in the field of sepsis, there is still no satisfactory diagnostic method for the rapid, robust, and sensitive identification of pathogens in the bloodstream. Due to lack of alternatives, time-consuming, contamination-prone blood cultures (BCs) are still considered the standard of care for patients with FN and sepsis [1, 8]. In addition, molecular detection methods are increasingly being used, but these assays are not comprehensive and detect only prespecified individual pathogens [7, 9, 10].

Metagenomic next-generation sequencing (mNGS) is a promising, comprehensive, hypothesis-free diagnostic approach that

has the potential to detect simultaneously multiple deoxyribonucleic acid (DNA) containing infectious agents with higher sensitivity and faster turnaround time than conventional methods [6, 7, 11–13]. Because analyzed pathogens do not have to be viable, detection is also independent of concurrent antibiotic treatment or culturability of microbes, and it also allows for discovery of translocated remnant DNA from focal infection sites such as the gut [14–16]. The overall analytical sensitivity of mNGS is influenced by the dominance of human host background cell-free DNA (cfDNA) where most reads, generally >99%, derive from the human host. However, this disadvantage, which is inherent to unbiased mNGS, could be mitigated either by targeted sequencing or host depletion methods [17].

DISQVER is a cell-free, DNA-based, mNGS pathogen detection test commercially available in Europe. DISQVER has an analysis time of 36 to 42 hours, including shipping time of up to 24 hours, and proved useful in patients with sepsis [4, 6]. In this study, we performed a 1-year, prospective, monocentric study involving hospitalized patients presenting with FN to evaluate the performance of DISQVER in comparison to conventional BC testing.

METHODS

Study Design
This was a prospective cohort study, enrolling patients with a high risk of developing FN, at the Division of Hematology, Medical University of Graz, Austria. High risk for FN was the eligibility criterion and defined as follows: pre-existing severe neutropenia; acute myeloid or lymphoblastic leukemia; high-dose chemotherapy for relapsed or refractory lymphoma; or hematopoietic stem cell transplantation (HSCT). The primary objective was the comparison of the diagnostic yield of the mNGS assay DISQVER (Noscendo GmbH, Duisburg, Germany) to established standard microbiological assays in patients with FN. Adult patients were enrolled before development of FN. Paired sample collection could be performed at any time point when blood culture was clinically indicated for FN by standard-of-care guidelines.

Neutropenia was defined as an absolute neutrophil count <500/µL or <1000/µL with high probability of decrease <500/µL in the next 2 days. Fever was defined as temperature >38.3°C once measured as well as >38.0°C with a duration of 1 hour or measured 2 times within 12 hours, respectively. Whole blood specimens were collected from central venous catheter ([CVC] ≥2 pairs aerobic/anaerobic BACTEC Plus bottles [BD Biosciences, Heidelberg, Germany]; 1 Streck blood collection tube; Cell-Free DNA BCT CE [Streck, La Vista, NE]) and peripheral vein ([PV] ≥1 pair aerobic/anaerobic BACTEC Plus bottles; 1 Streck blood collection tube) during FN episode for BC and mNGS analyses before initiation or change of antibiotics [18].

Paired BC and mNGS sampling were repeated at the earliest after 3 days of fever, which was defined as 1 FN episode. This time frame was chosen because American and German national infectious diseases (ID) society guidelines recommend to modify empiric antibiotic treatment in high-risk patients with persistent FN and documented infection after 2 to 4 days, including empirical addition of antifungal therapy after 72 to 96 hours [1, 19]. After analyzing 52 paired cfDNA samples, mNGS analysis was switched to only 1 sample, preferably from CVC, because the agreement between PV and CVC was high. The BC collection was continued as described.

Results of mNGS testing were not available during patient treatment and were not used to guide clinical treatment decisions. The sequencing team had no knowledge about clinical and microbiological results. All clinical data were retrospectively reviewed by 3 ID specialists (M.G., L.K., and T.V.) forming an ID review panel and 1 hematologist (E.S.) with experience in the treatment of infections after chemotherapy. Using the results of all clinical, imaging, and microbiological tests performed, the ID review panel used a composite approach to define cases in a binary fashion according to whether infection was unlikely versus proven, probable, or possible. In addition, the ID panel evaluated all infectious FN episodes analyzed by mNGS with respect to the 3 questions addressing antimicrobial therapy. (1) Was the initial antimicrobial treatment likely efficacious against the microorganisms detected by mNGS? (2) Were the microorganisms identified by mNGS possibly covered by the new empirical antibiotics? (3) Would a different antimicrobial treatment have been suggested if the mNGS result had been available in real time?

Blood Culture
All BC bottles were processed by standard procedures using the BACTEC automatic blood culture detection system (BD, Heidelberg, Germany) as previously described [20]. Subsequent identification of cultured microorganisms was performed by routine microbiological procedures including matrix-assisted laser desorption ionization time-of-flight mass spectrometry identification (Bruker Maldi Biotyper, Vienna, Austria).

Metagenomic Next-Generation Sequencing
Blood samples were drawn into Streck blood collection tubes and shipped at ambient temperature by a medical logistics integrator to the laboratories of Noscendo GmbH in Reutlingen, Germany. Blood samples were separated to plasma by centrifugation at 1600 ×g for 10 minutes at 4°C, and the plasma supernatant was transferred to a fresh reaction tube. Then, a second centrifugation step at 1600 ×g for 10 minutes at 4°C was performed, supernatants were again transferred, and plasma aliquots were further stored. Nucleic acid isolation, quality controls, and library preparation were carried out as previously described [21]. Adequate positive and negative controls

accompanied all laboratory and sequencing procedures. Raw sequencing data were subjected to various quality controls comprising PHRED-Score filtering, adapter trimming, complexity filtering, as well as k-mer-based contamination screening. To pass the quality filter, read quality needed to surpass a Phred score of 20 and achieve a minimal length of 50 base pairs after quality control. All data generated were analyzed using Noscendo's DISQVER platform, integrating the CE-IVD for pathogen detection assay from blood. 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 contamination, commensals from infective agents. Sequencing data can be retrieved from the European Nucleotide Archive (ENA) using accession number PRJEB54778.

Statistical Analysis

The analysis data set included all patients who developed FN according to predefined criteria at any time. Measures of agreement (percent positive agreement [PPA], percent negative agreement [PNA], percent overall agreement [POA]) between mNGS and BC and their exact binominal 2-sided 95% confidence intervals (CIs) were calculated using a 2×2 contingency table. McNemar's χ^2 test was used to test for difference of results between paired samples. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of mNGS and BC tests were calculated using MedCalc after clinical data review assigning the presence of a proven, probable, or possible infection versus unlikely infection.

Patient Consent Statement

The ethical committee of the Medical University of Graz (Graz, Austria) approved the study (vote 32-548 ex 19/20). The patient's written consent was obtained.

RESULTS

Patient Characteristics

Sixty-one patients developed FN at any time between September 2020 and July 2021 corresponding to a total of 98 FN episodes. Twenty-one patients (34.4%) had multiple sample collections either due to multiple hospital stays or persistent fever, and 15 (24.6%) of these patients had repeated sample collections within a window of at least 7 days. Patient characteristics are summarized in Table 1. The median age was 60 years (range, 30 to 77) and 44.3% (27 of 61) of patients were female. Acute myeloid leukemia ($n = 20$), non-Hodgkin lymphoma ($n = 13$) and multiple myeloma ($n = 11$) were the most common underlying cancer entities. Hematopoietic stem cell transplantation was the most common cause of neutropenia and accounted for 70.5% (43 of 61) of patients with FN. Antibiotics were

administered in 95.9% (94 of 98) of episodes at the time of sampling (Table 1).

Infections and Microbiologic Results

Sixty sources/sites of infection were documented in 45 patients during 98 FN episodes based on investigators' adjudication (Table 2). The most frequent sites of infections were mucositis and/or enterocolitis ($n = 29$), BSI ($n = 10$), and pneumonia ($n = 7$). The source of infection could not be determined in

Table 1. Patient Characteristics

Characteristics	N	%
Patients	…	…
Total	61	100
mNGS available	60	98
Male	34	56
Female	27	44
Age (years; median [range])	60 [30–77]	N/A
Diagnosis	…	…
Acute leukemia	25	41.0
Acute biphenotypic leukemia	2	3.3
Acute lymphoblastic leukemia	3	4.9
AML	20	32.8
Secondary AML	6	9.8
Therapy-related AML	2	3.8
MDS	4	6.6
Therapy-related MDS	1	1.6
Chronic myeloid leukemia	2	3.3
Primary myelofibrosis	1	1.6
Hodgkin-lymphoma	4	6.6
NHL	13	21.3
Diffuse large B-cell lymphoma	5	8.2
Follicular lymphoma	2	3.3
Mantle cell lymphoma	4	6.6
Peripheral T-cell lymphoma	2	3.3
Multiple myeloma	11	18.0
Testicular cancer	1	1.6
Therapy	…	…
Allogeneic hematopoietic stem cell transplantation	22	36.1
Autologous hematopoietic stem cell transplantation	21	34.4
Chimeric antigen receptor T-cell therapy	2	3.3
Acute leukemia—induction	7	11.5
Acute leukemia—consolidation	2	3.3
Acute leukemia—salvage	4	6.6
NHL induction	1	1.6
NHL salvage	1	1.6
None	1	1.6
Febrile episodes	…	…
Total	98	100
mNGS available	97	99
Antibiotics at the time of BC/mNGS sampling	…	…
Antibiotic treatment	54	55.1
Antibiotic prophylaxis	40	40.8
None or TMP/SMX thrice-weekly prophylaxis only	4	4.1

Abbreviations: AML, acute myeloid leukemia; BC, blood culture; BSI, blood stream infection; MDS, myelodysplastic syndrome; mNGS, metagenomic next-generation sequencing; N/A, not applicable; NHL, non-Hodgkin-lymphoma; SMX, sulfamethoxazole; TMP, trimethoprim.

8.1% (5 of 62). The BC positivity rate was 14.3% (14 of 98) of FN episodes (Table 3). Overall, 15 pathogens were detected in 12 patients, and *Pseudomonas aeruginosa* ($n = 4$) and *Staphylococcus epidermidis* ($n = 4$) were the most common (Table 3). It is interesting to note that in 10 of 15 (66.7%) detected bacteria, only 1 single BC bottle showed growth. One polymicrobial case was also noted. Here, *Staphylococcus hominis* was detected in 2 of 6 BC from the CVC (time to positivity [TTP] 7 and 15 hours in each of the positive BC bottles) and *Acinetobacter ursingii* in 1 of 6 BC (TTP 15 hours), respectively, hinting at contamination or a low microbial load. The BC from PV remained sterile for both bacteria.

Performance of Metagenomic Next-Generation Sequencing

The ID review panel adjudicated 66 (67.3%) of the 98 FN episodes as a result of an infection. In total, 97 (99%) FN episodes were successfully analyzed by mNGS of plasma cfDNA (Table 3). Metagenomic NGS testing failed in the BC-positive polymicrobial FN episode because of too low DNA content resulting from deep neutropenia and low bacterial burden as indicated by TTP of BC.

Overall, 43.3% (42 of 97) of all mNGS samples were positive, and 32% (31 of 97) were positive if single viruses were excluded. Focusing on all positive 42 mNGS samples and including polymicrobial results (Supplementary Figure 1 and Supplementary Table 2), the most frequent pathogens were coagulase-negative *Staphylococci* ($n = 10$), *Pseudomonas* ($n = 10$), and *Herpesviridae* ($n = 10$) followed by *Enterococci* ($n = 6$) and *Streptococci* ($n = 6$). Fungi were detected in 5% ($n = 5$) of FN episodes. It is notable that *Aspergillus oryzae* and *Aspergillus flavus* were determined by mNGS in the plasma of 1 patient with bronchioalveolar lavage (BAL) culture negative, probable invasive pulmonary aspergillosis diagnosed by positive galactomannan tests in serum as well as BAL.

Viruses were detected in 15 (15.5%) FN episodes including human herpesvirus 6 ($n = 7$), cytomegalovirus ($n = 2$), herpes simplex virus 1 ($n = 1$), and human adenovirus ($n = 1$), but only 4 (4.1%) were considered clinically relevant and a possible cause of fever (Table 2). As previously described, Torque teno virus was only detected in patients after allogeneic HSCT ($n = 4$) [22]. A conventional polymerase chain reaction (PCR) was performed in the temporal proximity of mNGS as part of standard microbiological tests in 10 of the 15 virus-positive FN episodes. The results of the conventional PCR agreed with the mNGS results in all 10 cases (Supplementary Table 1).

One fatal event occurred due to adenovirus infection or reactivation. A 76-year-old female patient with relapsed acute lymphoblastic leukemia developed BC-negative FN and liver complications (transaminitis, hyperbilirubinemia) after treatment with inotuzumab ozogamicin, vincristine, and steroids. Because veno-occlusive disease is a known major adverse event after treatment with inotuzumab ozogamicin and severe adenovirus infections are hardly known outside allogeneic HSCT, diagnosis was made delayed after more than 10 days by conventional PCR after other infectious and noninfectious causes were excluded. In this case, adenovirus had been detected by mNGS at first presentation of FN.

Table 2. Sources and Sites of Infections

Characteristic	N	%
All patients with FN	61	100
Patients with infections	45	73.8
All sources of infections	60	100
Adenovirus infection	1	1.7
Blood stream infection	10	16.7
Catheter-related BSI	4	6.7
Cholecystitis	1	1.7
CMV reactivation	1	1.7
Enterocolitis	6	10.0
HHV6 reactivation	2	3.3
Local catheter infection	1	1.7
Mucositis	23	38.3
Pneumonia	7	11.7
Probable invasive pulmonary aspergillosis	1	1.7
Possible invasive pulmonary aspergillosis	1	1.7
Sinusitis	1	1.7
Skin and soft tissue	1	1.7
Undefined	5	8.3
Urogenital	1	1.7

Abbreviations: BSI, blood stream infection; CMV, cytomegalovirus; FN, febrile neutropenia; HHV6, human herpesvirus 6.

NOTE: It is possible to have multiple sources/sites of infection per patient or FN episode.

Table 3. Febrile Neutropenia Episodes With Positive Results

Characteristic	N	%
Febrile neutropenia episodes	98	100
Blood culture positive	14	14.3
Acinetobacter ursingii[a,b]	1	…
Enterobacter cloacae[c]	1	…
Enterococcus faecium	2	…
Gemella haemolysans	1	…
Pseudomonas aeruginosa	4	…
Rothia mucilaginosa	1	…
Staphylococcus epidermidis[c,d]	4	…
Staphylococcus hominis[a,b]	1	…
mNGS available	97	99.0
Positive (all microorganisms)	42	43.3
Positive (excluding viruses only)	31	32.0
Positive (polymicrobial, ie, bacteria and/or fungi)	15	15.5

Abbreviations: mNGS, metagenomic next-generation sequencing.

NOTE: Two patients each had 2 febrile episodes with positive blood cultures.

[a]Same blood culture bottle.

[b]mNGS not available ($n = 1$).

[c]mNGS discordant with different pathogen ($n = 2$).

[d]Contamination probable ($n = 1$).

There were 73 identical and 24 discrepant results between mNGS and BC when focusing on at least 1 common pathogen in polymicrobial samples, corresponding to 75.3% (95% CI, 65.5% to 83.5%) agreement and 24.7% (95% CI, 16.5% to 34.5%) disagreement.

In contrast, positive and negative agreement for all pathogens between 52 paired mNGS from CVC and PV were markedly higher than between mNGS and BC. Given 47 identical and 5 discrepant results, agreement was 90.4% (95% CI, 79.0% to 96.8%) and disagreement was 9.6% (95% CI, 3.2% to 21.0%). Three of the 5 discrepant mNGS findings were due to reporting thresholds: species that were detected in 1 sample with read counts slightly over the reporting threshold were reported negative in the other paired sample due to variation at the detection limit. One discrepant result showed reproducibly *Burkholderia contaminans* only in the sample from the CVC suggesting contamination of the catheter.

Percent positive agreement, PNA, and POA between mNGS and BC were 84.6% (95% CI, 54.6 to 98.1), 63.1% (95% CI, 51.9 to 73.4), and 66% (95% CI, 55.7 to 75.3), respectively. The difference in diagnostic performance was statistically significant (Table 4).

We calculated sensitivity, specificity, PPV, and NPV of BC and mNGS for bacterial and fungal infections (Table 5). With similar NPV (40% vs 36.1%), mNGS showed 2-fold higher sensitivity (40% vs 18.5%) and higher accuracy (55.2% vs 43.8%) but—as expected—lower specificity (83.9% vs 96.8%) and lower PPV (83.9% vs 92.3%) compared to BC.

Consensus of Infectious Disease Specialists

According to the adjudication of the ID panel, no effective antimicrobial therapy was present in 21.5% (14 of 65), the pathogen was not covered by the new empiric antibiotic in 18.5% (12 of 65), and the antibiotic therapy would have been different in 26.2% (17 of 65) of FN episodes if the mNGS results had been available in real time. However, the adjudication process was limited because the ID review panel could not unambiguously answer the 3 research questions in 60% (39 of 65), 60% (39 of 65), and 62% (40 of 65) of the FN episodes, respectively. Reasons for failed adjudication were missing susceptibility data, negative mNGS despite presence of an infection

according to adjudication, and lack of unanimity between ID specialists.

DISCUSSION

Diagnosis and treatment of FN is challenging due to the low sensitivity of routine diagnostic tests and the need for immediate initiation of appropriate antimicrobial therapy. Because identification of the causative pathogen is not possible in most cases, treatment of patients might be longer than needed using broad-spectrum antibiotics and thus promoting the development of resistance. In other instances, inappropriate antibiotics may even have fatal consequences due to lack of efficacy or side effects. Thus, there is much room and need for improvement in the care of patients with FN. One promising unbiased approach is the use of mNGS for the detection of microorganisms complementing current standard microbiological tests.

To evaluate the performance of the mNGS test DISQVER in comparison to conventional BC testing, we performed a prospective single-center study of 61 hospitalized patients with FN predominantly after high-dose chemotherapy and HSCT. Most common sources of infection were mucositis, BSI, and pneumonia. Compared to BC (14% positive results), mNGS

Table 5. Diagnostic Test Evaluation of 96 FN Episodes With mNGS Results

Test system	Infection present/Statistics	Infection not present/Value	Total/95% CI
mNGS positive	26	5	31
mNGS negative	39	26	65
BC positive	12	1	13
BC negative	53	30	83
mNGS			
Sensitivity	40.0%	28.0% to 52.9%
Specificity	83.9%	66.3% to 94.6%
Positive Likelihood Ratio	2.5	1.1 to 5.8
Negative Likelihood Ratio	0.7	0.6 to 0.9
Disease prevalence	67.7%	57.4% to 76.9%
Positive Predictive Value	83.9%	68.8% to 92.5%
Negative Predictive Value	40.0%	34.1% to 46.2%
Accuracy	54.2%	43.7% to 64.4%
Blood culture			
Sensitivity	18.5%	9.9% to 30.0%
Specificity	96.8%	83.3% to 99.9%
Positive Likelihood Ratio	5.7	0.8 to 42.1
Negative Likelihood Ratio	0.8	0.7 to 0.1
Disease prevalence	67.7%	57.4% to 76.9%
Positive Predictive Value	92.3%	62.0% to 98.9%
Negative Predictive Value	36.1%	33.2% to 39.3%
Accuracy	43.8%	33.6% to 54.3%

Abbreviations: BC, blood culture; CI, confidence interval; FN, febrile neutropenia; mNGS, metagenomic next-generation sequencing.

NOTE: Viruses were excluded in the assessment of mNGS. One FN episode with negative mNGS and BC could not be adjudicated by the Infectious Diseases review panel, and 1 FN episode with a polymicrobial BC finding could not be successfully analyzed by mNGS.

Table 4. Comparison mNGS Versus Blood Culture

Test system	BC Positive	BC Negative	Total	P
mNGS positive	11[a]	31	42	…
mNGS negative	2	53	55	…
Total	13	84	97	<.001

Abbreviations: BC, blood culture; mNGS, metagenomic next-generation sequencing.

NOTE: One febrile neutropenia episode with a polymicrobial BC finding could not be successfully analyzed by mNGS (total analyzed positive BC, *n* = 13).

[a]Discordant with different pathogen (*n* = 2) compared to mNGS.

(43% positive results) was 3 times more likely to provide a possible microbiological etiology.

The unique feature of our study, in addition to the prospective design and high proportion of hematologic patients after HSCT, is that mNGS and BC samples were collected simultaneously and compared against each other. This is in contrast with other studies that (1) included immunocompromised patients without hematologic diseases and FN, (2) were retrospective in design, (3) allowed plasma collection for mNGS up to 24 hours of fever onset, or (4) compared mNGS to BC results generated within a window of 7 days [23–26].

The rate of antibiotic prophylaxis and treatment was high in our patient population at the time of sampling due to the underlying disease and the long-lasting neutropenia. The fact that patients could be analyzed at any time when conventional BC tests were indicated by standard of care resembles the real-world hospital setting where BC are repeatedly drawn in intractable or recurrent FN.

The detection rate of BC was 14% and very comparable to published as well as our own local microbiological surveillance data (data not shown) [6, 26]. Nevertheless, interpretation of BC was challenging especially in skin-colonizing bacteria because only single BC bottles showed growth in approximately two thirds of positive cases. In contrast, mNGS showed a high 90% agreement between paired plasma samples from CVC and PB. The false negativity rate of mNGS in patients with FN seemed to be dictated by a low DNA amount of pathogens. To increase sensitivity and detect pathogens near the detection limit of the assay, using paired specimens for mNGS similar to BC, where 3 pairs are considered standard, may be one useful approach in neutropenic patients until further improvements in sequencing and data analysis are achieved. However, costs and benefits must be weighed, because no test system will ever be perfect, especially in the absence of prospective interventional clinical trials demonstrating a clear advantage.

Other factors that might have led to decreased sensitivity of mNGS are ribonucleic acid viruses as cause of infection, sample collection at later timepoints of FN, and lack of pathogen transmission from certain sites of infection. As previously described, prevalence of viremia was high in our patient population, and agreement of mNGS with conventional virus PCR was 100% in cases where both tests were available [7]. However, our study was not designed to systematically compare test performance of mNGS with other virus tests.

Compared to BC, pathogen detection by mNGS resulted in a 2-fold higher sensitivity of 40% with a high PPA of 84.6%, a high PPV of 83.9%, and an overall higher accuracy. We were very conservative in the evaluation of our microbiological results and strictly adhered to the paired BC. Because we have a high suspicion but cannot be certain that at least 1 BC result was false positive, we suspect that sensitivity and specificity of

BC might be overestimated in our study. Still, the most frequent pathogens detected by mNGS in our cohort—coagulase-negative *Staphylococci*, *P aeruginosa*, *E faecium*—were also most prevalent in BC analysis.

The advantage of mNGS in comparison to BC is the ability to identify viral and fungal as well as polymicrobial infections particularly from the gastrointestinal tract during the neutropenic phase after chemotherapy [16]. In one publication, mNGS was able to reliably predict and diagnose the onset of bloodstream infection before onset of symptoms in cancer patients [27]. However, in another report of 167 asymptomatic patients undergoing measurement of microbial cfDNA sequencing, mNGS detected low-level (mostly apathogenic human commensals) organisms in 23%, whereas true infections displayed high-level concentrations pointing to putative difficulties in interpreting mNGS results in some test systems and underscores the importance of ID specialists [28].

The ID panel review determined that the antibiotic treatment should have been modified in 26% of FN episodes due to the mNGS findings. This shows the great potential of mNGS, which, however, cannot replace BC at this time because it cannot yet predict susceptibility and resistance to antibiotics. Because turnaround time of DISQVER is 36 to 42 hours, including shipping, routine use of mNGS as a complement to standard diagnostics comes within reach, especially in settings where sequencing can be performed onsite. However, interpretation of results and check for plausibility must necessarily be carried out together with experienced ID specialists. Further prospective interventional trials should be carried out to investigate effects of antibiotic escalation and de-escalation strategies on outcome parameters in patients with FN in the context of mNGS findings.

CONCLUSIONS

In summary, mNGS of plasma cfDNA using the DISQVER test detects microorganisms missed by conventional microbiological tests in patients with FN and may be a valuable addition to blood culture to guide antimicrobial therapy.

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