Evaluating Infectious, Neoplastic, Immunological, and Degenerative Diseases of the Central Nervous System with Cerebrospinal Fluid-Based Next-Generation Sequencing

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Abstract

Cerebrospinal fluid (CSF) is a clear and paucicellular fluid that circulates within the ventricular system and the subarachnoid space of the central nervous system (CNS), and diverse CNS disorders can impact its composition, volume, and flow. As conventional CSF testing suffers from suboptimal sensitivity, this review aimed to evaluate the role of next-generation sequencing (NGS) in the work-up of infectious, neoplastic, neuroimmunological, and neurodegenerative CNS diseases. Metagenomic NGS showed improved sensitivity—compared to traditional methods—to detect bacterial, viral, parasitic, and fungal infections, while the overall performance was maximized in some studies when all diagnostic modalities were used. In patients with primary CNS cancer, NGS findings in the CSF were largely concordant with the molecular signatures derived from tissue-based molecular analysis; of interest, additional mutations were identified in the CSF in some glioma studies, reflecting intratumoral heterogeneity. In patients with metastasis to the CNS, NGS facilitated diagnosis, prognosis, therapeutic management, and monitoring, exhibiting higher sensitivity than neuroimaging, cytology, and plasma-based molecular analysis. Although evidence is still rudimentary, NGS could enhance the diagnosis and pathogenetic understanding of multiple sclerosis in addition to Alzheimer and Parkinson disease. To conclude, NGS has shown potential to aid the research, facilitate the diagnostic approach, and improve the management outcomes of all the aforementioned CNS diseases. However, to establish its role in clinical practice, the clinical validity and utility of each NGS protocol should be determined. Lastly, as most evidence has been derived from small and retrospective studies, results from randomized control trials could be of significant value.

1 Introduction

Cerebrospinal fluid (CSF) is a clear and paucicellular fluid of low protein concentration, derived from the blood plasma and produced by the ependymal cells of the choroid plexus. In normal conditions, ependymal cells can produce around
500 mL/day of CSF, of which 150 mL circulates within the ventricular system and the subarachnoid space of the central nervous system (CNS) [1, 2]. Being a crucial component of the CNS structure, any change in the cellular/biochemical composition, volume, and flow of the CSF could affect CNS function, and CNS disorders may alter CSF characteristics [2]. These changes are caused by various diseases that impact the CNS either primarily or secondarily, while CSF examination often helps to identify the cause [1, 2].

Physicians sample CSF by performing a procedure called a lumbar puncture, where they insert a needle within the L3–L4 or L4–L5 intervertebral space of the patient, and subsequently send the samples to the laboratory for analysis [3]. Currently, the main diagnostic indications of CSF testing include confirming or excluding CNS infections that cause meningitis or meningoencephalitis, CNS primary or metastatic neoplasms, subarachnoid hemorrhage, and neuroinflammatory disorders such as multiple sclerosis (MS) [2, 3]. For instance, identifying numerous neutrophils in the CSF supports a bacterial infection, whereas malignant cells confirm the presence of a cancer disseminating within the subarachnoid space [1, 2].

Although vital for everyday clinical practice, conventional CSF testing has significant drawbacks, the most important of them being suboptimal sensitivity. Morphologic evaluation of infectious agents (e.g., using Gram stain), cultures, or serologic testing often leave patients undiagnosed, while polymerase chain reaction (PCR) testing needs a formal hypothesis for the potential infectious agents implicated to select the most suitable primers. As a result, antimicrobial treatment could cause delay or even be inappropriate, with significant impact on morbidity and mortality [4, 5]. Likewise, CSF cytology shows sensitivity of < 50% to detect cancer dissemination into the subarachnoid space, even if it is highly specific [6, 7]. While the presence of oligoclonal bands—which represent increased concentrations of IgG immunoglobulins—in the CSF characterizes most MS cases, these bands could also be detected in other inflammatory or even neoplastic CNS conditions [8]. Of interest, CSF examination could provide answers where other diagnostic modalities fail or may harm the patients; for instance, when a brain biopsy fails to diagnose and assess the molecular profile of a CNS tumor or it is contraindicated due to the tumor location or the condition of the patient’s health, CSF testing is a viable option [9].

In contrast to Sanger sequencing, next-generation sequencing (NGS) can process multiple nucleic acid fragments in parallel and within a single run, at both genomic (DNA) and transcriptomic levels (coding or noncoding RNA) [10]. Selection from various existing NGS-based methods depends on the clinical scenario. Targeted NGS using predefined panels is commonly applied in oncology [11], while metagenomic NGS (mNGS) is an emerging application in microbiology laboratories; mNGS is able to identify all pathogens present in a sample and in an unbiased way, without depending on a hypothesis, such as with PCR [12]. MicroRNA (miRNA) NGS can identify short, single-stranded, non-coding RNAs that regulate gene expression at the post-transcriptional level, and have acted as diagnostic and prognostic biomarkers in various disorders [13–15]. This review aims to highlight the value of applying NGS in the CSF of patients with infectious, neoplastic, neuroimmune, and neurodegenerative CNS diseases.

2 CNS Infections

Infections of the CNS—associated with bacteria, viruses, parasites, fungi, and prions—are a major cause of morbidity and mortality, especially in immunocompromised individuals [16, 17]. Based on the predominant anatomic site of infection, CNS diseases are usually classified as meningitis, encephalitis (or meningoencephalitis), or myelitis (or encephalomyelitis). Acute meningitis may be caused by bacteria or viruses, while fungi, mycobacteria, or atypical bacteria are usually involved in chronic meningitis [17]. The rapid identification of the pathogen is essential for the process of antimicrobial therapy and its impact on clinical outcomes [18]. More than 100 pathogens, commonly viruses, have been reported as causative agents of encephalitis; however, most of the cases remain undiagnosed or even underestimated and the selection of an appropriate diagnostic method must be the ultimate objective [19, 20].

It has been reported that in approximately 75–80% of pediatric CNS infections, microbiological diagnosis is usually obtained with molecular laboratory methods [21]. Recently, mNGS technology of the CSF has been suggested as a fast, sensitive, affordable but also costly and labor-intensive diagnostic tool, detecting unidentified pathogens in a single run, since culture, serologic procedures, and PCR have occasionally been proven ineffective [5, 16, 21–26]. This technology allows rapid sequencing of full pathogen genomes, even in point-of-care diagnostics, overcoming the limitations of available targeted PCR methods. It is useful in the examination of a large number of cases or small outbreaks, through accurate genotyping and molecular epidemiology, and also by characterization of genes mediating drug resistance [22, 27–29]. Interestingly, in a recent study, NGS of ancient dental calculus samples from a prehistoric case revealed high levels of Neisseria meningitidis and low levels of Haemophilus influenzae, which was interpreted as an ancient case of meningococcal disease associated with incipient endocranial lesions and pronounced meningeal grooves [30]. Nevertheless, clinical application is still in an early phase and there are several limitations [31] as most of the published reports consist of single case reports or small...
retrospective case series [32]. In addition, direct comparison between conventional testing and mNGS should rely on the definition of the gold standard method, further adding to the difficulty in validating such a new technology [31]. It has been shown that several background and contaminating bacteria (e.g., Propionibacterium, Burkholderia, Acinetobacter species) [33] and DNA contamination from CSF pleocytosis, a common feature in meningitis patients, considerably reduce the sensitivity of mNGS in the detection of pathogens [34]. Furthermore, several challenges including the improvement of bioinformatics software tailored for clinical diagnostic use and concerns over the quality and comprehensiveness of available reference databases still exist in making mNGS more applicable into laboratory practice [16]. Laboratory diagnosis of CNS infections with mNGS and the characteristics of the studies are summarized in Table 1 and Fig. 1.

2.1 Bacteria

Streptococcus pneumoniae followed by Streptococcus agalactiae, N. meningitidis, H. influenzae, and Listeria monocytogenes have been reported as the most common causes of acute bacterial meningitis [35]. Rapid diagnosis and treatment reduce mortality and neurological consequences, but this can be delayed by atypical clinical presentation, evaluation of the lumbar puncture safety, and poor sensitivity of conventional diagnostic methods [36]. Common pathogens including N. meningitidis, Streptococcus spp., L. monocytogenes, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae [26, 28, 37], but also rare opportunistic pathogens such as Psychrobacter spp. [38] have been successfully detected in CSF specimens of pediatric and adult patients. Zhang et al. analyzed the diagnostic value of mNGS for identifying S. pneumoniae in pediatric bacterial meningitis. Results obtained by mNGS were compared to those from CSF culture, which is recognized as the “gold standard” method [39], although culture-based methods are less sensitive than molecular techniques, because most patients usually receive antibiotics prior to admission [40]. The sensitivity and specificity of mNGS for detecting S. pneumoniae were 73.1% and 88.1%, respectively, whereas the positive predictive value (PPV) and negative predictive value (NPV) were 81.4% and 89.3%, respectively. In addition, the difference in number of unique reads of bacteria from CSF samples collected up to 14 days from disease onset was significantly higher than the pathogen load from the sample collected over 14 days from the clinical onset [39]. In another study, NGS was applied for the investigation of two N. meningitidis serogroup C isolates from a meningococcal outbreak, classified within the same N. meningitidis sequence type ST-11 by conventional molecular techniques. Results demonstrated subtle genetic differences among the above-mentioned isolates [28], providing useful information about the epidemic potential and dynamics of geographic distribution of distinct meningococcal serotypes [41].

L. monocytogenes is also an important bacterial cause of meningoencephalitis in immunocompromised individuals, with a high mortality rate. The positivity rates of Gram stain and CSF culture have been reported to be 14% and 41%, respectively, and NGS recently identified and sequenced L. monocytogenes from three cases of clinically suspected meningoencephalitis [26]. In another study, four patients with a clinically suspected CNS infection and non-specific clinical manifestations were diagnosed with neurobrucellosis using NGS of the CSF, while only one patient (25%) had a positive CSF culture. Furthermore, it is estimated that approximately 28% and 15% of patients with CNS brucellosis have positive blood cultures and CSF cultures, respectively, and although serological procedures are both more sensitive and faster than conventional culture, seropositivity is high in endemic areas and may not easily distinguish active from past infections [33, 42].

Infections with Mycobacterium tuberculosis (MTB) may occur with CNS comorbidities in approximately 1% of cases and are associated with significant mortality and adverse neurologic outcomes [17]. In a retrospective analysis, 51 patients with suspected tuberculous meningitis were examined with mNGS and four other assays in CSF. The sensitivity, specificity, PPV, and NPV of mNGS were 84.4%, 100%, 100%, and 46.1%, respectively, while sensitivity was significantly higher than that of acid-fast staining (0%), CSF culture (22.2%), MTB RT-PCR (24.4%), and Xpert MTB/RIF (40%) [43]. In another study, the sensitivity of mNGS, acid-fast staining, CSF culture, and MTB RT-PCR in the first CSF samples from patients with definite tuberculous meningitis were 66.7%, 33.3%, 8.3%, and 25%, respectively, whereas the specificity of all procedures was 100% [44]. Lately, mNGS has also been evaluated for Ureaplasma parvum detection in the CSF of a neonate with meningitis [45].

2.2 Viruses

In more than half of cases, the etiological cause of CNS viral diseases remains unknown. Non-specific culture assays, serological tests (e.g., ELISA) and molecular techniques with high sensitivity and specificity (e.g., quantitative PCR) have been used as diagnostic tools, but they are compromised by several limitations since they are targeted against individual viruses or subsets of similar viruses [24]. NGS seems to be a promising method for the diagnosis of both DNA and RNA viral infections as it enables the detection and identification of known as well as novel pathogens [46, 47]. Although viruses in terms of genome size may be small, and their viral load may be undetectable due to the high concentration of background nucleic acids, viral metagenomics
| First author, year | Specimen (cases (n)) | Age | Pathogen identified (diagnosis) | Comments | Reference |
|-------------------|----------------------|-----|--------------------------------|----------|-----------|
| Edridge et al. 2019 | CSF (n=47) | Adult population | Enterovirus, HIV-1, VZV (meningitis, encephalitis) | Capacity to detect low load RNA viruses | [24] |
| Kawada et al. 2016 | CSF (n=18) | 3 mo–15 year | Coxsackievirus A9 mumps virus (acute encephalitis, encephalopathy) | Difficult to detect herpesvirus DNA (compared with qPCR) | [25] |
| Miller et al. 2019 | CSF (n=95) | Adult population | Several bacteria, viruses, parasites and fungi (meningitis, encephalitis) | Costly and labor intense | [16] |
| Yao et al. 2016 | CSF (n=3) | 40–66 year | L. monocytogenes (meningoencephalitis) | Highly sensitive | [26] |
| Wilson et al. 2019 | CSF (n=204) | 0–>60 year | Several bacteria, viruses and fungi (meningitis, encephalitis, myelitis) | The overall accuracy for pathogen detection relative to conventional methods was 90%, with 73% clinical sensitivity and 99% specificity | [32] |
| Ji et al. 2020 | CSF (n=20) | 21–82 year | MTB C. neoformans (meningitis) | Reduction of human DNA contamination can effectively improve the sensitivity of pathogen detection | [34] |
| Ortiz-Alcantara et al. 2016 | CSF (n=1) | Pediatric patient | Psychrobacter spp. (meningitis) | mNGS was a powerful tool to identify a rare unknown pathogen in a clinical case of meningitis | [38] |
| Zhang et al. 2019 | CSF (n=135) | 5.9–33.5 mo | S. pneumoniae (meningitis) | Compared with conventional tests (culture and antigen detection), the sensitivity and specificity of mNGS for S. pneumoniae identification were 70.3 and 93.9%, respectively | [39] |
| Yan et al. 2020 | CSF (n=51) | Median age 34 year | MTB (meningitis) | The diagnostic sensitivity of mNGS was 84.4% | [43] |
| Wang et al. 2019 | CSF (n=23) | 3–73 year | MTB (meningitis) | Combination of mNGS and conventional methods increased the detection rate up to 95.6% | [44] |
| Wang et al. 2020 (45) | CSF (n=1) | 11-day-old infant | U. parvum (meningitis) | Quick and timely diagnosis, successful treatment without complication | [45] |
| Saha et al. 2019 | CSF (n=91) | 0–160 mo | Several bacteria and viruses (infectious and idiopathic meningitis) | mNGS can be used as a complementary tool in endemic areas and outbreaks | [48] |
| Wang et al. 2018 | CSF (n=1) | 42-year | N. fowleri (meningoencephalitis) | Early detection, effective treatment | [49] |
| Fan et al. 2018 | CSF (n=4) | 31–58 year | T. solium (neurocysticercosis) | Background or contaminating bacteria do not influence the interpretation of NGS results when determining CNS parasitic infections | [50] |
| Fei et al. 2020 | CSF (n=7) | 36–66 year | T. solium (neurocysticercosis) | mNGS may successfully be used for the clinical diagnosis of NCC, since this mainly depends on neuroimaging and immune diagnostic procedures | [51] |
| Xie et al. 2019 (54) | CSF (n=2) | 15–22 mo | Angiostrongylus cantonensis (eosinophilic meningoencephalitis) | A. cantonensis-induced eosinophilic meningoencephalitis, a rare parasitic disease in infants can be precisely diagnosed by mNGS | [54] |
| Xing et al. 2019 | CSF (n=12) | 15–68 year | Cryptococcus neoformans/Cryptococcus gattii species complexes (meningitis) | Due to the limitations of conventional techniques, mNGS may serve as a potential alternative diagnostic tool. | [57] |

CSF cerebrospinal fluid, CNS central nervous system, NGS next-generation sequencing, mNGS metagenomic next-generation sequencing, qPCR quantitative polymerase chain reaction, HIV-1 human immunodeficiency virus–1, VZV varicella-zoster virus, MTB mycobacterium tuberculosis
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overcomes this by the enrichment of the viral genomic content in the sample [24].

Herpes viruses, measles virus, Coxsackieviruses, and Polyomaviruses have been identified by mNGS from CSF, pooled nucleic acids from CNS specimens and brain biopsies as known causes of encephalitis. Arenaviruses, Astroviruses, Bornaviruses, Coronaviruses, and Parvoviruses are considered novel causes of encephalitis. Cycloviruses, Densoviruses, and Gemycircularviruses have been isolated as unexpected pathogens from clinical specimens, demonstrating an unclear association with the reported infections [22]. Especially in low- and middle-income countries, unbiased mNGS can complement conventional diagnostic methods in order to identify potentially pathogenic viruses that cause epidemics, such as Chikungunya virus and as well as other RNA viruses, but also may be useful for the conduct of surveillance studies, and prediction of outbreaks [48]. Although techniques to reduce host DNA contamination from CSF samples are still lacking, several effective approaches have been used in order to improve the detection sensitivity of pathogens [34]. Using negative controls with predetermined DNA composition has been shown to be a necessary option to define the expected microbial nucleic acid background [29]. Nevertheless, mNGS should be performed in combination with conventional methods in order to diagnose a viral infection in patients presenting with mild-to-moderate lymphocytic pleocytosis [32].

2.3 Parasites

In contrast to bacterial and viral infections, laboratory diagnosis of some parasitic infections cannot be primarily accomplished by conventional methods. Parasites are more frequently found in immunocompromised than immunocompetent patients with meningoencephalitis, while the most common CNS protozoan infection in the former is Toxoplasma gondii [17]. In a recent study, toxoplasmic encephalitis with atypical brain imaging features and negative results of Toxoplasma IgM antibodies test, was rapidly diagnosed by an mNGS method, in a critically ill...
HIV-infected individual [19]. In addition, NGS technology was successfully used in the accurate diagnosis of primary amoebic meningoencephalitis, an acute and life-threatening disease caused by Naegleria fowleri [49].

Regarding helminths, neurocysticercosis caused by Taenia solium is the most common infection of the CNS. The clinical manifestations and MRI findings may vary due to several factors, such as the number, stage, size, and different locations of the encysted larvae, thus the diagnosis usually becomes quite challenging. Also, considering the variable PPV of the available serological experiments, detection of T. solium DNA in CSF with NGS could facilitate diagnosis [50, 51]. In such a case, a patient who presented for several months with atypical clinical symptoms including headache and paroxysmal left face and arm numbness, was diagnosed with neurocysticercosis using NGS in the CSF [52]. Furthermore, when compared with CNS bacterial infections, the NGS results for neurocysticercosis are readily interpreted, since they are not influenced by the existing background or contaminating bacteria [50]. Angiostrongylus cantonensis is the most common parasitic nematode cause of eosinophilic meningitis. Since the diagnosis of angiostrongyliasis also lacks standardized and confirmatory assays including serological tests and CSF cytology, the development of a precise diagnostic method is a challenge. A recent report has described a case of angiostrongyliasis confirmed by NGS in the CSF of a patient with ELISA-negative results [53]. Similarly, NGS was considered to have higher sensitivity and specificity when compared to microscopy, serological tests, and MRI in the detection of A. cantonensis eosinophilic meningoencephalitis in two infants [54].

2.4 Fungi

Fungal CNS infections with either yeasts or molds can cause severe neurological manifestations of either meningitis or focal abscess and cause life-threatening complications in the immunocompromised host. Most of these pathogens are acquired through the respiratory tract and secondarily disseminate through the hematogenous route to the CNS [17]. Among fungal infections, the opportunistic infection with Cryptococcus neoformans/Cryptococcus gattii species complexes has become the leading cause of meningitis among HIV-infected patients and is associated with high morbidity and mortality rates [55, 56]. mNGS has been successfully applied as an important diagnostic tool for rapid and specific diagnosis of cryptococcal infection in CSF samples, especially for accurate species identification, in conjunction with the traditional diagnostic methods including India ink staining, CSF culture, and cryptococcal antigen detection [57]. Recently, mNGS has been reported as a more sensitive procedure than conventional CSF culture and cryptococcal antigen test for the detection of Cryptococcus spp. at even low abundance in CSF of HIV-infected individuals. In this study, three HIV-seropositive, cryptococcal antigenemic patients with clinical features of meningitis and negative CSF microbiological procedures were finally diagnosed with cryptococcal meningitis by mNGS [58]. Diverse fungal pathogens including members of the genera Aspergillus, Histoplasma, and Candida have been also successfully identified by NGS technology [59].

3 CNS Cancers

As most primary and metastatic tumors involving the CNS show poor prognosis, their prompt diagnosis along with effective personalized therapy and close follow-up are imperative [60–62]. To this end, tumor molecular profiling is increasingly used in the clinic as an integral part of precision oncology [63, 64]. Molecular tumor data are often collected with tissue-based testing, either small biopsy or resection specimens [62]. However, invasive CNS procedures could carry significant risk, especially when sampled in patients with medical comorbidities or from critical areas such as the brainstem, prohibiting serial testing to monitor tumor evolution or treatment response. Furthermore, brain tumors might be inaccessible to biopsy, while, if reachable, the tissue specimens could be inadequate, show hypocellularity, abundant necrosis, or poor preservation that hamper subsequent molecular analysis [62, 65]. Of interest, both primary and metastatic CNS tumors display significant intra-tumor heterogeneity. This means that processing a small biopsy might not represent the whole tumor. Likewise, molecular testing of a primary extracranial cancer could be insufficient to guide treatment decisions for its CNS metastasis [9, 63, 66, 67].

Liquid molecular analysis could facilitate tumor diagnosis, monitoring, and therapy selection, and overcome inherent problems related to a tissue biopsy. Similar to tissues, cancer mutational testing can be performed in body fluids such as the blood, urine, sputum, pleural fluid, and CSF [9, 68–70]. Even if it often fails to reach the diagnostic capacity of its tissue counterpart and lacks sufficient evidence of clinical validity and utility, the liquid biopsy is minimally invasive, cheaper than tissue biopsy, easier to obtain, and free from preservatives and fixatives. In addition, it depicts intra-tumor heterogeneity effectively and allows tumor follow-up with serial testing [69, 71, 72]. Any liquid counterpart including circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), non-coding RNAs (e.g., miRNAs), or exosomes can be analyzed with diverse molecular techniques [69, 73]. Of the latter, NGS is especially appealing, as it offers the capacity to examine comprehensively multiple cancer-related molecular alterations (point mutations,
| First author, year | CNS tumor types/number (n) of patients involved in the analysis | NGS type | Main findings | Reference |
|-------------------|---------------------------------------------------------------|----------|---------------|-----------|
| Zhao et al. 2020  | Primary (glioblastoma, diffuse astrocytoma, anaplastic astrocytoma, anaplastic oligodendroglioma)/n=17 | DNA-Seq  | NGS in CSF showed high concordance with tumor tissue molecular analysis, albeit it revealed additional molecular alterations; lesions in proximity to the CSF space were more likely to show ctDNA in their CSF molecular analysis | [82] |
| Li et al. 2019    | Primary (glioblastoma)/n=5 | DNA-Seq  | NGS in CSF monitored CNS tumor molecular evolution more accurately than in plasma | [83] |
| Pan et al. 2019   | Primary (brainstem glioma)/n=57 | DNA-Seq  | NGS in CSF identified alterations in most cases and showed high concordance with tumor tissue molecular analysis, while it revealed additional alterations; CSF ctDNA revealed higher mutation detection rate than plasma ctDNA; IDH1-mutant cancers exhibited favorable, whereas H3F3A/HIST1H3B-mutant cancers poor prognosis | [203] |
| Martínez-Ricarte et al. 2018  | Primary (diffuse glioma)/n=20 | DNA-Seq  | NGS in CSF facilitated molecular diagnosis and classification of most diffuse gliomas tested, while sensitivity dropped in low-grade tumors or in lesions non-adjacent to the CSF space | [81] |
| Miller et al. 2019 | Primary (diffuse glioma)/n=85 | DNA-Seq  | NGS in CSF revealed ctDNA in about half of the patients, while its presence was associated with dismal prognosis; the mutational landscape of CSF ctDNA was highly concordant with that of tumor tissue analysis, though it revealed additional alterations that reflected tumor heterogeneity and evolution; NGS in CSF showed higher mutation detection rate than in plasma | [84] |
| Mouliere et al. 2018 | Primary (glioblastoma, oligodendroglioma, others)/n=13 | DNA-Seq  | Whole genome sequencing was used to detect ctDNA in CSF and identified copy number variations in 38% of the cases; ctDNA concentration was dependent on tumor grading and location (tumors of high-grade or adjacent to the CSF space released more ctDNA) | [87] |
| Wang et al. 2015  | Primary (glioblastoma, medulloblastoma, anaplastic astrocytoma, others)/n= 35 | DNA-Seq  | NGS in CSF revealed ctDNA in 74% of the cases, while its detection was dependent on tumor grading (high-grade tumors shed more ctDNA) and location (tumors adjacent to the CSF space released more ctDNA) | [88] |
| Duan et al. 2020  | Primary (glioblastoma)/n=10 | DNA-Seq  | NGS in CSF aided the molecular diagnosis and classification of glioblastomas, while it also detected additional alterations from tumor tissue analysis | [85] |
| Grommes et al. 2019 | Primary and secondary CNS lymphomas/n=15 | DNA-Seq  | NGS in CSF facilitated disease follow-up and monitored response to therapy, while it detected ctDNA even when imaging or CSF cytology were negative | [204] |
| Zhao et al. 2020  | Primary (glioblastoma) and metastatic (lung, gastric, breast, etc.) cancers/n=58 (62 CSF samples) | DNA-Seq  | All samples tested revealed mutations, the most common of which were TP53, EGFR, PTEN, CDKN2A; CNVs of CDKN2A, CDK4, and MDM2 were the most common ones detected; EGFR mutations found in CSF were highly concordant with matched tissue molecular analysis (in 9/10 patients) | [205] |
| Baumgarten et al. 2020 | Primary (glioblastoma, CNS lymphoma) and metastatic (breast, lung, others)/n=27 | DNA and RNA-Seq  | NGS in CSF identified alterations in the majority of the tested cases, while some mutations were targetable guiding cancer treatment; a number of cases revealed increased TMB, which is known to be predictive of response to immune checkpoint inhibitors; NGS in CSF showed higher diagnostic sensitivity than imaging or CSF cytology | [62] |
| Kopkova et al. 2019 | Primary (glioblastoma, low-grade glioma, meningioma) and metastatic/n=70 | RNA-Seq  | NGS in CSF revealed differences in the miRNA levels of the tested CNS tumors compared to their controls | [111] |
| First author, year | CNS tumor types/number (n) of patients involved in the analysis | NGS type | Main findings | Reference |
|--------------------|---------------------------------------------------------------|----------|---------------|-----------|
| Pentsova et al. 2016 | Primary (glioblastoma, anaplastic oligodendroglioma, others) and metastatic (NSCLC, breast, melanoma, others)/n=53 (44 with and 9 without CNS involvement) | DNA-Seq | NGS in CSF detected actionable alterations and identified mechanisms of therapy resistance (e.g., EGFR T790M mutation), while none of the nine samples with no evidence of CNS involvement revealed any mutation; NGS in CSF showed higher diagnostic sensitivity than CSF cytology; ctDNA and mutation detection rate was higher in CSF cytology positive than negative cases. | [96] |
| De Mattos-Arruda et al. 2015 | Primary (glioblastoma, medulloblastoma) and metastatic (lung, breast)/n=12 | DNA-Seq | NGS in CSF showed higher concentration of ctDNA in patients with CNS restricted disease and higher mutation detection rate than in plasma, while it was more representative of the CNS tumors’ molecular profile; NGS in CSF also facilitated diagnosis and monitored tumor evolution and response to treatment. | [107] |
| Pan et al. 2015 | Primary (meningioma, Schwannoma) and metastatic (lung, melanoma, breast, others)/n=8 | DNA-Seq | CSF molecular analysis with NGS and/or digital PCR revealed mutations in 7/8 cases tested; ctDNA concentration was higher in CSF than in plasma in patients with low tumor systemic tumor burden. | [86] |
| Li et al. 2020 | Metastatic (NSCLC)/n=94 | DNA-Seq | EGFR mutations were found in 79/94 of the advanced lung adenocarcinoma patients tested, while the presence of CDK4, TP53, MET, CDKN2A, MYC, and SMAD4 mutations was associated with poor prognosis. | [206] |
| Xing et al. 2020 | Metastatic (NSCLC)/n=38 | DNA-Seq | In this prospective study (APOLLO trial), significant molecular heterogeneity was found between CSF and plasma-based NGS testing; two patients with EGFR-T790M in the CSF at baseline responded to osimertinib treatment; the quantity of EGFR-T790M was reduced after a 6-week treatment and patients exhibited a trend towards improved progression free survival. | [197] |
| Zheng et al. 2020 | Metastatic (NSCLC)/n=45 (cohort 1) and 35 (cohort 2) | DNA-Seq | In cohort 1, patients treated with osimertinib exhibited differences in progression free survival based on the EGFR mutation detected, while the coexistence of CD4 or CDKN2A with EGFR mutations was associated with shorter survival; in cohort 2, potential resistance mechanisms to osimertinib were detected, such as the C797S mutation. | [198] |
| Miao et al. 2020 | Metastatic (NSCLC)/n=23 | DNA-Seq | NGS in CSF revealed EGFR mutations in two patients who exhibited EGFR-wild type in tissue-based molecular analysis. | [207] |
| Shen et al., 2020 | Metastatic (NSCLC)/n=1 | DNA-Seq | NGS in CSF identified the mechanism of anti-EGFR therapy resistance (EGFR T790M mutation). | [106] |
| Ma et al. 2020 | Metastatic (NSCLC)/n=21 | DNA-Seq | NGS in CSF identified uncommon EGFR mutations that guided cancer treatment decisions. | [101] |
| Ma et al. 2020 | Metastatic (NSCLC)/n=21 | DNA-Seq | NGS in CSF identified targetable alterations (e.g., EGFR mutations) in the majority of the tested cases; the mutation detection rate was higher in CSF than in plasma and concordant to tumor tissue analysis. | [100] |
| Ma et al. 2019 | Metastatic (NSCLC)/n=1 | DNA-Seq | NGS in CSF identified an uncommon EGFR mutation (G719A) that guided cancer treatment. | [208] |
| Liu et al. 2019 | Metastatic (NSCLC)/n=1 | DNA-Seq | NGS in CSF identified the mechanism of anti-EGFR therapy resistance (EGFR T790M and L718Q mutations). | [105] |
| Zhao et al. 2019 | Metastatic (lung, gastric, breast, others)/n=35 | DNA-Seq | NGS in CSF showed higher diagnostic sensitivity than imaging or CSF cytology. | [98] |
| Aldea et al. 2020 | Metastatic (NSCLC)/n=12 | DNA-Seq | NGS in CSF showed higher mutation detection rate than in plasma. | [112] |
insertions, deletions, copy number variations, and gene rearrangements) in a single run [74].

This section describes the recent evidence regarding CSF-based NGS testing in patients with primary or metastatic CNS tumors. We summarize this evidence in Table 2 and illustrate its importance in Fig. 2.

### 3.1 Primary CNS Cancers

Regarding primary CNS cancers, GLOBOCAN estimated 296,851 (1.6% of all sites) new cases and 241,037 deaths (2.5% of all sites) globally in 2018 [75]. Gliomas represent the most common primary CNS malignancy, while...
glioblastomas account for most gliomas in the adults, exhibiting low resectability and high recurrence rates [64]. In children, CNS tumors follow leukemias in incidence yet they lead all pediatric cancers in morbidity and mortality; of them, the embryonal tumor medulloblastoma is the most common malignant lesion [76]. Diagnosis and management of primary CNS tumors largely depend on their grade. Grading measures diverse tumor characteristics including cellular atypia, mitoses, necrosis, and endothelial proliferation. The higher the grade, the worse the prognosis generally is; for instance, glioblastomas and medulloblastomas are both grade IV tumors of dismal prognosis [60].

Molecular profiling of CNS tumors is essential for their accurate diagnosis and should be performed together with morphologic evaluation, according to the latest WHO classification [60]. Gliomas are classified as IDH-mutant and IDH-wild type, as their prognosis differs significantly, being worse in the wild type. In addition, 1p/19q co-deletion is a molecular alteration characterizing oligodendrogliomas, while a diffuse glioma of lower grade (II or III) and an IDH-wild type status is upgraded to “molecular glioblastoma” (integrated grade IV glioma) when alterations such as EGFR amplification or TERT promoter alterations are found [60, 64, 77]. TERT promoter mutation is associated with a dismal prognosis, while MGMT promoter methylation is associated with a favorable response to chemotherapy and prolonged survival [64, 78, 79]. Likewise, hypermutation after chemotherapy is a major factor of tumor progression and therapy resistance [64, 80].

CSF-based molecular analysis in primary CNS tumor patients could provide valuable information concerning accurate diagnosis and classification, alterations linked with prognosis and therapy selection, tumor evolution, and response to treatment (Table 2). Besides testing 20 tissue biopsies and CSFs from glioma patients, Martinez-Ricarte et al. also analyzed 648 diffuse gliomas from the TCGA Atlas and developed an NGS platform that can genotype seven genes (including IDH1, IDH2, TP53, TERT, and H3F3A); this platform could facilitate the classification of gliomas with CSF ctDNA, avoiding the risk of invasive sampling [81]. Zhao et al. applied targeted NGS and identified driver mutations from the CSF ctDNA in both newly diagnosed and relapsed glioma patients; of interest, the concordance rate with their matched tumor tissue samples in this study was high [82]. As CSF sampling is minimally invasive, CSF-derived ctDNA analysis is not only promising for defining gliomas’ initial molecular signature but also for subsequent serial testing to monitor potential tumor progression [83, 84].

Concerning the value of NGS as an element of CSF molecular analysis, several teams have compared the molecular signatures of the CSF-derived ctDNA to the tissue-derived DNA. In brief, CSF-based NGS analysis was largely concordant with the tissue-based analysis. Furthermore, it also spotted additional mutations, reflecting intratumoral heterogeneity, while it overcame the under-sampling bias inherent to small biopsies [9, 82, 84–86]. NGS diagnostic capacity was directly correlated to the tumor grade and location. High-grade tumors (e.g., glioblastomas) with a location next to the CSF space were more likely to excrete ctDNA into the CSF circulation [81, 82, 87, 88]. Likewise, NGS in CSF exhibited superior performance to plasma molecular analysis, revealing higher mutation detection rates [83, 84, 86].

3.2 Metastatic CNS Cancers

Metastatic spread is a frequent complication and substantial cause of mortality in patients with a cancer that first develops outside the CNS, most commonly in the lung, breast, or skin (melanoma). Prognosis in such patients is generally poor, hence there is an emerging need to integrate molecular data into personalized treatment strategies [27]. Recent guidelines recommend that patients with advanced non-small-cell lung cancer (NSCLC) should undergo predictive biomarker testing for potential EGFR, ALK, ROS1, BRAF molecular alterations, as targeted therapies exist for them. In addition, mutations such as the EGFR T790M are associated with resistance to therapy [63, 89, 90]. Likewise, patients with breast cancer and melanoma should be tested for the presence of HER2 amplification or BRAF V600E mutation to assess their prognosis and evaluate their eligibility for trastuzumab or the BRAF inhibitor vemurafenib, respectively [63, 91]. While delivery of traditional chemotherapy into the CNS lesion is significantly limited by the blood-brain barrier, targeted therapies seem to outweigh chemotherapy in terms of efficacy and safety [62, 63]. As the metastatic cancer molecular profile might differ from its primary counterpart, testing of only the primary site could be insufficient as it ignores heterogeneity and tumor evolution. In fact, the European Association of Neuro-Oncology recommends that, even if a cancer is previously assessed in its primary extracranial location, predictive biomarker testing should also be performed in the CNS metastatic site [62, 63, 91, 92].

Similar to primary CNS tumors, the application of CSF-based NGS testing in metastatic tumors could also facilitate diagnosis and prognosis, identify targetable mutations, assess intra-tumor heterogeneity, monitor tumor evolution and therapy response, and identify mechanisms of drug resistance (Table 2). Diagnosis and follow-up of metastatic CNS lesions are typically performed with neuroimaging and CSF cytology; however, the latter suffer from low sensitivity [93, 94]. NGS was found to outweigh the diagnostic performance of imaging and cytology in terms of sensitivity for the detection of brain metastases [62, 95–98]. It was
also able to detect targetable mutations in metastatic lung (e.g., EGFR, ALK, or ROS1) [62, 96, 97, 99–102], melanoma [95–97], and breast cancer [96], also identify mechanisms of acquired resistance to therapy, such as the EGFR T790M mutation [96, 103–106]. Von Baumgarten et al. tested the CSF of 27 patients with metastatic and primary CNS tumors and reported that, by applying NGS to analyze the CSF-derived ctDNA, they could identify targetable mutations and change the treatment strategy in some of their patients. They also identified six cases with several molecular alterations suggesting high tumor mutational burden (TMB), which is known to be predictive of response to immune checkpoint inhibitors [62]. Many groups have also suggested the potential value of CSF-based NGS testing to follow-up patients with brain metastases and evaluate their response to treatment or identify disease progression, which could be spotted earlier compared to clinical presentation, imaging, or CSF cytology [42, 107–110]. While most studies have used ctDNA for their NGS experiments, Kopkova et al. demonstrated differences in the miRNA levels of the CNS tumors compared to controls by examining the CSF samples of 70 patients with both metastatic and primary lesions [111].

Similar to primary CNS tumors, CSF-based NGS testing was also largely concordant with tissue molecular analysis in metastatic CNS cancer patients, while it further detected additional mutations that were potentially missed due to tissue under-sampling. [9, 42, 100, 102–104]. Likewise, NGS in CSF was more sensitive than plasma molecular analysis in metastatic cancer patients [86, 97, 100, 102, 104, 107, 109, 110, 112, 113].

### 4 Neuroimmunological and Neurodegenerative Disorders

Genetics of neuro-psychiatric disorders involve diseases in the CNS controlled by either monogenic inheritance or complex polygenic inheritance and associated with an interplay of genetic risk and environmental influence [114, 115]. The rapid advancement of molecular testing during the last decades with NGS and genome-wide association studies (GWAS) has revolutionized the area, facilitating the research of disease mechanisms and enhancing the accuracy of diagnosis. Genetic studies in neurologic and psychiatric
disorders are focused on the investigation of the genome, the transcriptome, or the epigenome. While for disorders with monogenic type of inheritance, testing for genomic variations in the peripheral blood is adequate, for diseases with complex polygenic inheritance transcriptome and epigenome analysis is equally important [116]. Brain tissue from autopsy or biopsy and peripheral blood are the most commonly used sources for this kind of testing, but during recent years the CSF has also been investigated [117]. To date, the CSF has been used as a source of genetic material for NGS studies, mainly for transcriptomics in autoimmune neurological disorders and neurodegenerative disorders. The characteristics of these studies are summarized in Table 3 and Fig. 3. NGS has also been used in CSF of patients with acute ischemic stroke and several brain-enriched miRNAs have been detected 3 days after stroke, mostly associated with large ischemic strokes [118]. Finally, in recent years, the genetic basis of psychiatric disorders has begun to be identified [116, 119–122], but the only NGS study in the CSF contacted so far is related to the detection of known and novel viruses in patients with schizophrenia [123].

4.1 Neuroimmunological Disorders

Multiple sclerosis (MS) is the most common autoimmune disease of the CNS with still unknown etiology. The interaction between genetic risk factors and environmental influence is believed to be the key feature of the disease pathogenesis [124]. Initial genetic studies revealed strong associations in MS for genes within the major histocompatibility complex (MHC) including the human leukocyte antigen (HLA) class I and II [125–127]. More recently, during the last two decades, the advent of GWASs revealed more than 200 genetic risk variants for MS located in introns or exons, most of which reside in non-MHC regions [128–131]. The functional consequences of these variants are not yet fully understood, apart from a small number that are proven to be implicated in the regulation of the immune system [132–135]. On the way to better characterize the pathogenetic cascade that is related to the genetic risk variants, transcription and epigenetic mechanisms have been investigated as well, adding meaningful information to pathway analysis [135–139]. To this extent, the genetic material isolated from CSF, tested with NGS, provides valuable information both for research and for diagnostic purposes.

B and T cells have a central role in the pathogenesis of MS, as they interact and infiltrate the CNS causing local pathology and demyelination [140]. Lymphocytes of MS patients seem to undergo extensive clonal expansion inside the CNS [141, 142], but there is still a long debate about the mechanisms and the location of B- and T-cell activation, the invasion of CNS, their maturation and their reactivation. Findings of NGS in B and T cells of the peripheral blood, the CSF and the brain lesions have largely contributed to the understanding of the disease pathogenesis. Initial NGS studies on the repertoire of IgG heavy chain variable region genes showed that B cells may experience ongoing stimulation and maturation in the CNS, but they exchange across the blood-brain barrier and they can be identified in the peripheral blood [141, 143, 144]. Another NGS study, however, claimed that most of their maturation takes place outside the CNS in the draining cervical lymph nodes [145]. Further NGS analysis of CSF-derived B cells from MS patients has shown rearranged IgG variable heavy chain family 4 genes, suggestive of pronounced affinity maturation in the CNS, a feature that can be used as a biomarker for the diagnosis of MS [146–149]. In line with these findings, other studies revealed persistence over time of intrathecal oligoclonal B cells and IgG [150] and intrathecal somatic hypermutation of IgM chains that are not observed in the peripheral blood [151]. Similarly, for T cells, initial NGS studies have shown that CD8+ and CD4+ cells isolated from brain autopsy lesions can be also found in the CSF [152], and that clonally expanded CD8+ T cells are located in brain lesions, the CSF, and the peripheral blood [153]. However, further high-throughput sequencing of the T-cell receptor repertoires of the CSF revealed that they are largely distinct from the blood in MS patients [154, 155], and that there is intrathecal enrichment of EBV-reactive CD8+ T cells and CD8+CD161+ T cells [156, 157]. Interestingly, NGS of CD8+ and CD4+ T-cell receptor gene repertoires from CSF was used recently to support the diagnosis of MS [158] and to test the efficacy of treatment with autologous hematopoietic stem cell transplantation [159]. Furthermore, recently single-cell transcriptomics revealed increased transcriptional diversity in blood, and increased cell-type diversity in CSF including a higher abundance of cytotoxic phenotype T helper cells clonally expanded B cell in MS [160, 161].

Neuromyelitis optica (NMO) is a rare autoimmune disease of the CNS characterized by inflammatory response against aquaporin-4 (AQP4). A recent study comparing NGS analysis of B cells in the CSF and the peripheral blood revealed that CSF AQP4-specific B cells are closely related to an expanded population of double negative B cells of the peripheral blood that may undergo maturation within the CNS [162].

4.2 Neurodegenerative Disorders

During the past few decades, tremendous progress on the understanding of neurodegenerative diseases has been achieved. However, neurodegenerative diseases constitute a heterogenic cluster with various degrees of overlapping in terms of clinical features, imaging findings, serum and CSF parameters, pathological characteristics, and associated genes [163–168]. While pathological brain changes
### Table 3  Characteristics of NGS studies in CSF in neuroimmunological and neurodegenerative diseases

| First author, year | Disease/cases (n) | NGS type | Main findings | Reference |
|--------------------|-------------------|----------|---------------|-----------|
| Bankoti et al. 2014 | MS (n=5) | RNA-Seq | Oligoclonal bands are not merely the terminal result of a targeted immune response in MS but represent a component of active B cell immunity that is dynamically supported on both sides of the blood-brain barrier | [143] |
| Palanichamy et al. 2014 | MS (n=8) | RNA-Seq | Identified peripheral memory B cells, plasma cells/plasmablasts, and B cells that had an immune connection to the CNS compartment | [144] |
| von Büdingen et al. 2012 | MS (n=6) | RNA-Seq | Identified clonally related B cells that participate in robust bidirectional exchange across the blood-brain barrier | [141] |
| Stern et al. 2014 | MS (n=5) | DNA-Seq | The majority of the B cells associated with MS maturation takes place outside the CNS | [145] |
| Rounds et al. 2014 | MS (n=8) | DNA-Seq | A pattern of mutations in the antibody genes of CSF B cells can identify patients who have RRMS and patients who will convert to RRMS | [146] |
| Rounds et al. 2015 | MS (n=13) | DNA-Seq | VcH gene mutations of CSF B cells can identify patients with RRMS | [147] |
| Johansen et al. 2015 | MS (n=10) | DNA-Seq, RNA-Seq | Immunoglobulin heavy-chain variable gene transcripts were identified in blood B cells and clonally related CSF B cells | [148] |
| Ostmeyer et al. 2017 | MS (n=71) | DNA-Seq | VcH gene mutations of CSF B cells can be used as a statistical classifier in the diagnosis of RRMS | [149] |
| Tomescu-Baciu et al. 2019 | MS (n=2) | RNA-Seq | Persistence overtime of intrathecal oligoclonal B cells and IgG | [150] |
| Beltrán et al. 2014 | MS (n=12) | RNA-Seq | Intrathecal somatic hypermutation of IgM chains that are not observed in the peripheral blood | [151] |
| Planas et al. 2015 | MS (n=1) | RNA-Seq | CD8+ and CD4+ cells isolated from brain autopsy lesions can be also found in the CSF | [152] |
| Salou et al. 2015 | MS (n=7) | RNA-Seq | Clonally expanded CD8+ T cells are located in brain lesions, the CSF and the peripheral blood | [153] |
| Lossius et al. 2014 | MS (n=10) | RNA-Seq | There is intrathecal enrichment of EBV-reactive CD8+ T cells and CD8+CD161 T cells | [156] |
| Nicol et al. 2018 | MS (n=90) | RNA-Seq | There is intrathecal enrichment of EBV-reactive CD8+ T cells and CD8+CD161 T cells | [157] |
| Gerdes et al. 2016 | MS (n=1) | RNA-Seq | NGS of CD8+ and CD4+ T-cell receptor genes repertoires from CSF may be used to support the diagnosis of MS | [158] |
| Harris et al. 2020 | MS (n=24) | RNA-Seq | NGS of CD8+ and CD4+ T-cell receptor genes repertoires from CSF may be used to test the efficacy of treatment with autologous hematopoietic stem cell transplantation | [159] |
| Schafflick et al. 2020 | MS (n=60) | RNA-Seq | Increased cell type diversity in CSF, including abundance of cytotoxic phenotype T helper cells in MS | [160] |
| Ramesh et al. 2020 | MS (n=12) | RNA-Seq | Clonally expanded B cell in MS were associated with inflammation, blood–brain barrier breakdown, and intrathecal Ig synthesis. | [161] |
| Kowarik et al. 2017 | NMOSD (n=7) | DNA-Seq | AQP4-specific B cells are closely related to an expanded population of double negative B cells of the peripheral blood that may undergo maturation within the CNS | [162] |
| Ostmeyer et al. 2017 | MS (n=71) | DNA-Seq | VcH gene mutations of CSF B cells can be used as a statistical classifier in the diagnosis of RRMS | [149] |
| Jain et al. 2019 | AD (n=17) | RNA-Seq | miRNAs and piRNAs are associated with the progression of MCI to AD | [70] |
| Burgos et al. 2014 | PD (n=57), AD (n=62) | RNA-Seq | miRNAs from the periphery and the CSF may be used to predict disease progression | [23] |
| Hossein-Nezhad et al. 2016 | PD (n=27) | RNA-Seq | CSF miRNAs related to PARK7, LRRK2, SNCA genes could be used as potential biomarkers in PD | [65] |
| Otake et al. 2019 | ALS (n=4) | RNA-Seq | Presence of miRNAs of genes associated with ubiquitin-proteasome, unfolded protein and oxidative stress pathways in CSF of ALS patients | [120] |

**NGS** next-generation sequencing, **CSF** cerebrospinal fluid, **MS** multiple sclerosis, **RRMS** relapsing-remitting multiple sclerosis, **EBV** Epstein-Barr virus, **NMOSD** neuromyelitis optica spectrum disorders, **AQP4** aquaporin-4, **AD** Alzheimer disease, **miRNA** microRNA, **EOAD** early-onset AD, **MCI** mild cognitive impairment, **piRNA** PIWI-interacting RNA, **PD** Parkinson disease, **ALS** amyotrophic lateral sclerosis

△ Adis
may begin up to 30 years before the symptomatic phase in some of them [169, 170], only symptomatic treatments exist, all trying to counterbalance the neurotransmitter disorganization and none to hamper disease progression [171]. Furthermore, symptomatic treatments are effective only in the early disease stages [172]. Thus, early detection is crucial, but accurate diagnosis is a real challenge for the clinical neurologist, and it is not uncommon for the diagnosis to be established only post-mortem [173].

Alzheimer disease (AD) is the most common neurodegenerative disease. While AD is characterized by an age-related pathology, there are a variety of genes related to early-onset AD [174]. Among them, the most common is Allele 4 of apolipoprotein E (ApoE4), which is also the most important risk factor for sporadic AD [175]. The pathological hallmarks of AD are amyloid plaques and neurofibrillary tangles in brain tissue, reflected by a decrease in amyloid-β peptide 1–42 (Aβ42), and increase in total Tau and phosphorylated Tau protein (p-tau) in CSF [176]. Aβ42 is considered to be responsible for triggering a downstream that leads to Tau accumulation within neurons and, eventually, apoptosis [177]. Nevertheless, several other factors are implicated in AD pathogenesis, including mitochondrial, metabolic, inflammatory, and oxidative stress mechanisms [178–181]. Recently, genetic studies in the CSF significantly contributed to the clarification of these pathways. MicroRNAs (miRNAs) seem to play an important role in abnormal gene expression for genes associated with AD pathology [174]. During the past few years, several studies have identified with RT-PCR numerous miRNAs implicated in AD pathogenesis, some of them detected in the CSF [182–185]. Additionally, transcriptome analysis of the CSF with NGS in AD managed to identify a variety of miRNAs and other small noncoding RNAs considered to be candidate biomarkers for AD, while changes in miRNA expression were shown to correlate well with aspects of disease severity [186, 187].

Parkinson disease (PD) is the second most common neurodegenerative disease. It is characterized by motor symptoms, including bradykinesia, rigidity, and tremor, along with non-motor symptoms [169]. Its pathological hallmark is the progressive neuronal loss in the substantia nigra pars compacta due to accumulation of protein α-synuclein into cytoplasmic inclusions [188]. From there, the pathology spreads to the basal ganglia and the brain cortex. This could explain the variety of motor and non-motor symptoms associated with PD, including REM-sleep behavior disorder, dysautonomia, depression, and, finally, dementia. Thus, similar to AD patients, PD patients also seem to express
cognitive impairment, especially in the late stages of the disease, when advanced neurodegeneration has taken place [165, 166]. This common disease progression, at least in the late stages of the PD, implies that AD and PD might share some common pathological features [167, 168]. So, the initiative of transcriptomics in AD has also motivated similar studies in PD. Burgos et al. compared miRNAs from the periphery and the CSF from patients with PD or AD and normal controls [187]. They found miRNA dysregulation in both AD and PD patients compared to normal controls. Interestingly, some of the miRNAs were differentially expressed in both PD and AD, while others were expressed only in AD or PD patients. After that, a few other miRNAs in the CSF have been identified as potential PD biomarkers. Some of them were related to genes that are associated with PD, such PARK7, LRRK2, and SNCA [189].

Amyotrophic lateral sclerosis (ALS) is another neurodegenerative disease, whose main characteristic is the progressive loss of upper and lower motor neurons, commonly in association with cognitive and behavioral symptoms [190]. Indeed, an association between ALS and certain types of frontotemporal dementia has been well established [164]. Otake et al., using NGS transcriptome technology, analyzed exosomal mRNAs in human CSF [191]. In comparison with CSF of normal controls, 543 genes were found to have mutations and most of them were involved in the ubiquitin-proteasome, unfolded protein, and oxidative stress pathways.

5 Discussion

Several studies suggest that CSF-based NGS has a role in the evaluation of infectious and neoplastic CNS diseases, while evidence concerning immunological and neurodegenerative diseases is at a more rudimentary level. NGS is a high-throughput, fast, and sensitive, albeit labor-intensive and costly, technique able to process multiple nucleic acid fragments in parallel and within a single run, at both genomic (DNA) or transcriptomic levels, without requiring prior knowledge of the exact nucleic acid sequence(s). This contrasts with PCR applications, which entail a hypothesis of potential targets in order to select the most suitable primers [10, 192]. Of interest, most CSF-based next-generation transcriptomic analyses applying RNA-Seq have been reported in MS, AD, or PD studies (Table 3), whereas only a minority of oncology studies have utilized this methodology instead of DNA-Seq (Table 2). Targeted NGS is often used in oncological applications [11], and mNGS in microbiological applications [12]. The latter could especially be of value when conventional, less sensitive microbiologic methods—such as morphologic evaluation (e.g., with Gram stain), cultures, or serologic testing—fail to diagnose the cause of a CNS infection, delaying treatment and impacting clinical outcome [4, 5]. In addition, because of its high-throughput nature, mNGS could help characterize genes responsible for antimicrobial drug resistance [29] or identify pathogens causing outbreaks [28]. However, despite its reported high diagnostic accuracy, mNGS clinical application is still at an early phase and there are several limitations [31], while most evidence has been derived from small retrospective studies [32].

The application of NGS in the CSF can facilitate diagnosis and classification, prognosis, selection of therapy, and follow-up of primary and metastatic CNS tumors (Fig. 1). Of interest, CSF liquid biopsy could supplement or even replace tissue-based molecular analysis in the right clinical setting (e.g., patients with severe comorbidities, CNS tumors in critical or inaccessible locations, indication for sequential sampling to monitor evolution or therapy response), as it minimizes operational risk, is cheaper, and provides molecular signatures concordant to tissue analysis [9, 62, 73]. In addition, CSF liquid biopsy does not suffer from the inherent problems of tissue biopsy that commonly hamper subsequent molecular analysis, such as inadequacy and formalin fixation, and could depict intra-tumor heterogeneity more comprehensively, especially when compared to small biopsies [62, 63, 66, 193]. Likewise, it exhibits higher sensitivity to detect leptomeningeal metastases than imaging or CSF cytology [62, 95–98]. Evidence suggests that CSF liquid biopsy should be favored over its plasma counterpart when evaluating a CNS tumor, either primary or metastatic, as it shows a higher concentration of ctDNA and a higher mutation detection rate, and it depicts the CNS lesion molecular profile more accurately, especially when dealing with brain metastases. Plasma liquid biopsy exhibits reduced diagnostic capacity since the blood-brain-barrier hampers the shedding of CNS tumor-derived genetic material into the blood. In addition, as blood contains more normal circulating DNA derived from its higher population of non-malignant cells than the paucicellular CSF, ctDNA comprises only a small fraction of the total circulating DNA, resulting in lower sensitivity of the NGS assay [9, 62, 73, 84, 96, 97].

In the era of personalized oncology, laboratories are often asked to test minimal samples (such as the CSF) for multiple cancer biomarkers to assess patients’ eligibility for targeted therapies. To this end, NGS is an attractive option, as it allows high-throughput testing in a single run saving at the same time precious diagnostic material [74, 89, 194–196]. Notably, NGS is an excellent tool to identify potential mechanisms of therapy resistance, such as the EGFR-T790M or C797S in metastatic NSCLC, in addition to concurrent alterations associated with shorter survival [197, 198], or high TMB, which is known to be predictive of response to immune checkpoint inhibitors [62]. Although existing evidence seems promising, this is mostly derived from small and retrospective studies of low statistical power (similar to
infectious diseases). Concerning future directions, relevant NGS oncology assays should ideally be designed specifically for CSF and validated at both pre-analytical and analytical levels [9]. In addition, as most published studies describing CSF-based molecular analysis have tested ctDNA (Table 2), it would be interesting to see more studies researching DNA methylation, miRNAs [111], or exosomes. Research directed on the molecular subtypes of diverse primary or metastatic cancers such as glioblastoma [199] or breast cancer [200] could potentially improve the clinical management and patient outcomes.

As far as neuroimmunological and neurodegenerative diseases are concerned, NGS in the CSF is still in an early stage. CSF-based, single-cell NGS at genomic and transcriptomic levels has added valuable mechanistic insight for the understanding and the diagnosis of MS [201]. Compartmentalization between blood and CSF and maturation of B and T cells have extensively been studied in MS, while NGS in the CSF has offered significant help for our understanding about the development and the progression of this disease [145, 153, 154, 157]. During the past two decades there was a boom in the use of genomics and transcriptomics for the study of neurodegenerative diseases [202]. However, a limited number of studies has been performed so far with NGS in the CSF of patients with AD, PD, and ALS, evaluating the potential role of miRNAs as biomarkers for disease progression. Despite their promising results, use of miRNAs as a routine examination in the CSF is still impractical, not only due to the cost but also their still undetermined significance [186, 187, 189, 191]. Moreover, studies that include other common neurodegenerative diseases, such as Frontotemporal and Lewy body dementias, are still lacking. Hence, further investigation is needed towards a wider spectrum of neurodegenerative diseases sorting out their most significant and common findings.

In conclusion, clinical application of NGS in the CSF of patients with CNS diseases can help physicians make accurate diagnoses, thus planning treatments effectively and reducing adverse effects and mortality rates. Metagenomic NGS has shown improved sensitivity—compared to traditional microbiological methods—to detect bacterial, viral, parasitic, and fungal infections, while overall diagnostic performance could be maximized when all modalities were applied. In patients with primary CNS cancers, NGS findings in the CSF were largely concordant with the molecular signatures derived from tissue-based molecular analysis, showing the potential to supplement and even replace tissue-based molecular profiling in the right clinical context, while additional mutations were detected in the CSF in some studies, reflecting intratumoral heterogeneity. In patients with metastatic CNS cancers, NGS could facilitate diagnosis and classification, prognosis, therapy selection, and follow-up, exhibiting higher sensitivity than neuroimaging, cytology, or plasma molecular analysis. Although most evidence has derived from studies dealing with infectious or neoplastic CNS disorders, NGS has shown potential to help diagnosing or understanding the pathogenesis of MS and neurodegenerative diseases, such as AD, PD, and ALS. However, as all aforementioned applications are still in an early phase, validation of NGS for each clinical scenario at both pre-analytical and analytical levels is vital. Lastly, as most evidence stems from small and retrospective series, studies in the form of randomized controlled trials would be of paramount importance to establish clinical validity and utility.

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