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Review 综 述

宏基因组测序在中枢神经系统感染性疾病诊断中的应用及研究进展

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摘　要：中枢神经系统(central nervous system，CNS)感染是指由病毒、细菌或真菌等侵染中枢神经系统引起的急性或慢性炎症性(或非炎症性)疾病，致死率高，易引发严重后遗症。由于检测通量及灵敏度的限制，一半以上的中枢神经系统感染患者无法通过常规检测方法确定病原体。宏基因组测序是一种新兴的病原检测技术，能够极大地提升病原检出率。当前部分临床医生及相关从业人员对宏基因组测序的认识存在不足，限制了其在临床诊疗中的快速推广和应用。本文系统介绍了宏基因组测序整体流程，综述了该技术在中枢神经系统感染性疾病诊疗中的发展历程和最新研究进展，希望为中枢神经系统感染性疾病的诊断和治疗提供参考。

关键词：宏基因组测序；中枢神经系统感染；脑脊液；病原检测

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Metagenomic next-generation sequencing in diagnosis of infectious diseases of central nervous system

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Abstract: Infections of central nervous system (CNS) are acute or chronic inflammatory (or non-inflammatory) diseases caused by the invasion of viruses, bacteria, or fungi in CNS. Infectious diseases of CNS have high mortality rate with serious sequelae. Due to the limitation of detection strategies and low sensitivity, the pathogens of more than half of patients with infectious diseases of CNS cannot be identified by traditional methods. Metagenomic next-generation sequencing (mNGS) is a new technology and can significantly improve the detection rate of pathogens. However, mNGS is still not well understood by a few clinicians and other related people, which limits its rapid promotion and application in clinical diagnosis and treatment. This review systematically introduced the whole process of mNGS, and summarized the development history and the latest research progress of mNGS in the diagnosis of infectious diseases of CNS, thereby providing references for the diagnosis and treatment of infectious diseases of CNS.

Keywords: metagenomic next-generation sequencing; infections of central nervous system; cerebrospinal fluid; pathogenic detection

中枢神经系统感染性疾病主要包括脑炎、脑膜炎和脊髓炎等。脑炎多由病毒感染引起，常见于儿童，可引起严重后遗症，其中最常见的症状包括发育迟缓(35.0%)、行为异常(18.0%)和智力缺陷(17.5%)等[1]。脑膜炎主要分为无菌性和细菌性两类。前者较为常见，通常是自限性的且预后良好；后者具有起病急、死亡率高且后遗症严重的特点，需及时明确感染病原体以对症治疗[2]。脊髓炎可引起髓鞘或轴突损伤，致使患者丧失感觉功能甚至瘫痪等[3]。近期针对我国急性脑膜炎或脑炎患者(acute meningitis or encephalitis，AME)持续 10 年的纵向监测结果显示：AME 易感于儿童，病毒性病原体是造成患儿死亡的主要原因之一[4]。我国 CNS 感染患者中常见的病原体也是病毒。新发、再发和耐药病原体导致的病因不明及多重感染的病例数日益增加，进一步增大了临床病原诊断难度。因此，开发快速、灵敏且无偏倚的病原检测技术对中枢神经系统感染性疾病的精准诊疗具有重要意义。

1 宏基因组测序

宏基因组测序(metagenomic next-generation sequencing，mNGS)是一种基于下一代测序技术的新型病原检测技术。相较于传统检测技术，其能更加快速、准确和高通量地识别和分型病原[5]。mNGS 检测目标覆盖了大量已知基因组序列的病原且可持续纳入新发现的病原。图 1 概括了 mNGS 技术应用于临床诊断的发展历程并节选了国内外部分驱动该技术发展的关键事件。mNGS 最初被应用于环境微生态研究[6]。2014 年，

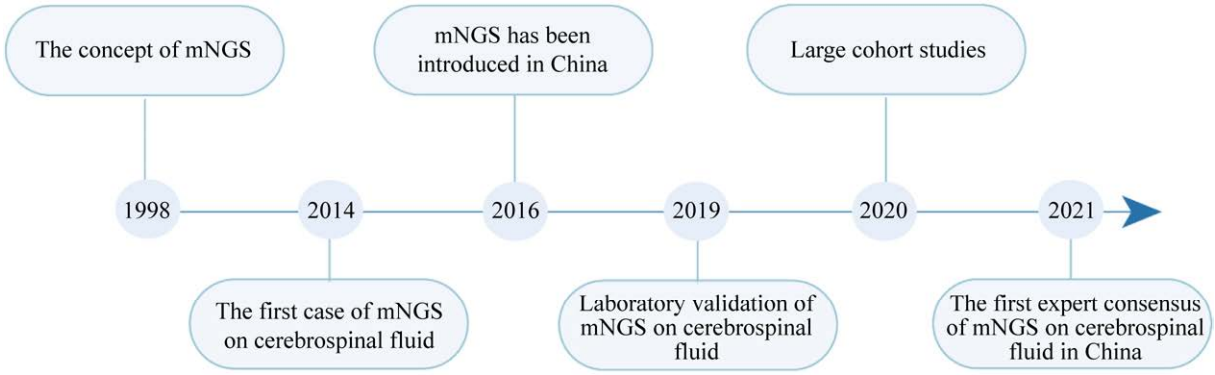

图 1　mNGS 技术应用于中枢神经系统感染性疾病诊断的发展历程

Figure 1　Application of mNGS in the diagnosis of infectious diseases in central nervous system.

Wilson 等利用 mNGS 确定了一名病因不明、反复发热及免疫缺陷症的脑炎患者的病因是钩端螺旋体感染[7]。此事件正式拉开了 mNGS 应用于临床检测的序幕。2016 年，mNGS 技术在国内进入应用阶段[8]。2019 年，Miller 等开发一套适用于 CNS 感染的 mNGS 检测流程，其实验室也通过了临床实验室改进法案修正案(Clinical Laboratory Improvement Amendments，CLIA)的认证[9]。同年，Wilson 等领衔的多中心脑脊液样本研究全面地揭示了 mNGS 在病原鉴定、耐药基因预测、疾病动态监测和演化分析中的潜在应用价值[10]。近期我国自主开展的多项队列研究再次证实了 mNGS 技术巨大的病原检测潜力，与传统方法相结合可有效地提高病原检出率[11–14]。2021 年，吴钢等撰写了《中枢神经系统感染性疾病的脑脊液宏基因组学第二代测序应用专家共识》[15]。这是国内首篇关于脑脊液 mNGS 临床应用的专家共识，为 CNS 感染性疾病精准诊断提供了重要的帮助。

2　mNGS 实验及数据分析

2.1　mNGS 实验流程与相关技术

2.1.1　样本处理

脑脊液(cerebrospinal fluid，CSF)是诊断 CNS 感染性疾病的主要样本。由于病原载量较低，脑脊液样本 mNGS 检测的灵敏性和特异性极易受背景菌和污染菌等的影响[16]。因此，待检患者临床样本的采集、运输及检测等全过程必须避免污染。若采集后 4 h 内进行检测，脑脊液样本可在 2–8 °C 条件下运输和储存。若存储时长低于 1 周，可将样本储存于–20 °C。预计保存时长超过 1 周的样本应储存于–70 °C 冰箱，运输过程需全程干冰。需长期保存的样本还应加入 RNA 稳定剂以抑制核酸降解。《中枢神经系统感染性疾病的脑脊液宏基因组学第二代测序应用专家共识》探讨了适用于 mNGS 检测的脑脊液样本采集要求，也具有重要的参考价值[15]。

2.1.2　核酸提取

病原核酸的有效提取是开展 mNGS 检测的前提，因此应设计预实验验证核酸提取方案是否有效。一般提取方法无法有效破壁的病原体，如结核杆菌和真菌等，珠打、煮沸等额外处理步骤可有效提升核酸提取率以避免假阴性检测结果。实验过程还应引入阳性和阴性对照组对整体流程进行质量控制(图 2)。阳性对照常使用已知病原微生物等的预混液；阴性对照可使用核酸提取试剂洗脱缓冲液等[17]。

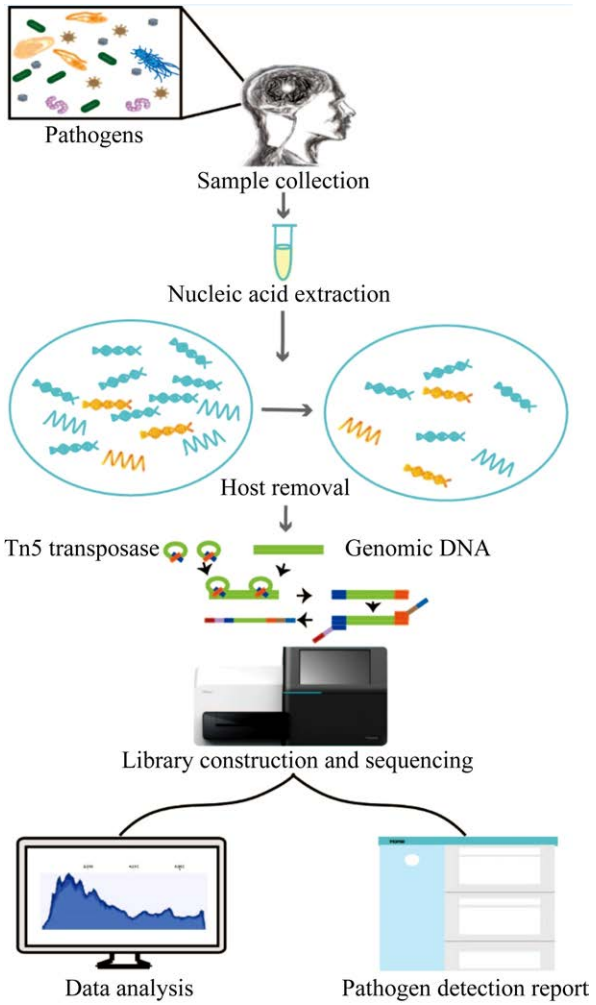

图 2　mNGS 检测流程示意图
Figure 2　mNGS workflow.

2.1.3　人源核酸去除

脑脊液中人源核酸占比较高严重降低了病原的检出率，直接进行测序可能导致假阴性检测结果。因此在测序文库构建之前，人源核酸应被尽可能地去除[16]。常用的去人源 DNA 技术有：离心、过滤和差异裂解等。离心法操作简单且成本较低。Gu 等采用 16 000 r/min 离心 10 min 去除脑脊液中人源细胞沉淀[18]。过滤法可以滤除颗粒较大的细胞和真菌等，适合于富集病毒。Kawada 等采用 0.45 μm 过滤器去除脑脊液中的人源细胞[19]。差异裂解法可选择性裂解游离人源细胞 DNA。Simner 等在脑脊液中加入皂苷裂解人源细胞并用 DNase 消化游离的人源 DNA[20]。RNA 建库还需额外注意以下事项：总 RNA 需使用 DNase 处理以除去残留的 DNA 污染[9]。上述方案均存在一定的局限性，实际操作可考虑综合应用多种方案以获得理想的去宿主核酸效果。此外总 RNA 中约 80%–90%为核糖体 RNA[21]，可使用探针杂交[22]和 CRISPR-Cas9[23]等方法去除宿主核糖体 RNA。

2.1.4　测序文库构建

脑脊液测序文库构建包括核酸片段化、接头连接、扩增及纯化 4 个主要步骤。RNA 需在文库构建前完成逆转录和二链合成步骤。核酸总量需满足构建测序文库的最低要求。总核酸一般采用酶切或机械打断方式进行片段化。其中转座酶打断的方式要求的起始核酸量较低，适用于脑脊液样本建库。Nextera XT DNA Library Preparation Kit 等被广泛应用于构建脑脊液宏基因组测序文库[9–10]。文库质检合格后进行双端或单端测序。脑脊液样本宏基因组测序数据量通常不应低于 20 000 000 测序读长(reads)[24]。

2.2　数据分析

mNGS 数据分析主要包括质量控制、人源序列去除、序列组装、物种鉴定及耐药和毒力基因分析等。测序读长可用 Fastp[25]和 Trimmomatic[26]等工具进行质量控制。质控合格的 reads 可使用 BWA[27]或 Bowtie2[28]等工具将其比对到人类参考基因组。比对结果可通过 Samtools[29]等工具滤除人源序列。剩余的 reads 使用 SPAdes[30]及 Megahit[31]等工具进行不依赖于参考基因的 *de novo* 组装，也可使用 CLC Genomics Workbench (QIAGEN)等图形化分析工具进行参考基因组依赖的一致性序列(consensus sequence)组装。组装出的序列可借助 BLAST[32]

等工具与微生物数据库进行比对。常用的病原数据库有美国生物技术信息中心(National Center for Biotechnology Information，NCBI)的核酸序列数据库(Nucleotide Sequence Database，NT)和参考序列数据库(Reference Sequence Database，Refseq)、临床级微生物数据库(Food and Drug Administration-database for Regulatory Grade Microbial Sequences，FDA-ARGOS)、全球微生物数据中心(World Data Center for Microorganisms，WDCM)和基因组分类学数据库(Genome Taxonomy Database，GTDB)等。此外，Kraken 等工具采用了基于 Kmer 匹配的算法[33]，可快速根据原始测序数据鉴定样本中微生物的种属。耐药和毒力基因分析一般使用 PathoFact[34]等工具，将组装序列与公共参考数据库比较进行注释。相关参考数据库包括毒力因子数据库(Virulence Factors Database，VFDB)和抗性基因数据库(Antibiotic Resistance Genes Database，ARDB)等。

当前 mNGS 数据分析尚缺乏标准化流程[35]，病原微生物基因组数据库也不尽完善。同一测序数据不同分析流程的分析结果间可能存在一定差异。因此，在搭建生物信息分析平台时应进行充分地调试，严格引入质量控制体系以保证生成合理且稳定的病原鉴定结果[36]。最后，针对不同种类的病原应设置适应的阈值判断标准，报告解读时还须注意排除污染菌与试剂工程菌等干扰。数据分析相关流程的搭建也可参考其他相关综述[35–37]。

3 mNGS 应用于 CNS 感染性疾病诊断研究进展

3.1 mNGS 利于检出脑脊液中罕见和未知病原体

mNGS 在检测新发和罕见病原方面存在显著优势。基于 mNGS 技术，大量未知的引起人中枢神经系统感染的病原体被不断地鉴定出来，如松鼠博尔纳病毒[38]和伪狂犬病病毒等[39–42]。相较于传统检测方法，mNGS 技术更有利于罕见病原的检测，如嗜冷杆菌[43]、解脲支原体[44]、广州管圆线虫[45–46]和猕猴 α 疱疹病毒 1 型[47]等均可被 mNGS 检出。此外，脑脊液 mNGS 检测为疑难危重 CNS 感染病患提供快速精准的诊疗依据，有利于协助临床医生合理地使用药物[48]。

3.2 mNGS 在临床诊断中的重要应用价值和巨大发展前景

近期多个研究中心开展的针对 CNS 感染性疾病的大规模队列研究，证实 mNGS 技术在临床诊疗和公共卫生领域具有重大应用价值。相对于传统检测方法，mNGS 具有较高的敏感性[49]，不易受抗生素影响且能在短时间内进行大量病原体的筛查鉴定[13]。其强大的病原检出能力可有效排除感染性病因，帮助自身免疫性和肿瘤等患者及时接受精准治疗[50]。针对特定病原体的队列研究表明，mNGS 对结核性脑膜炎诊断的灵敏度明显高于分枝杆菌生长指示管培养、改良 Ziehl-Neelsen 染色和 Xpert MTB/RIF 方法，可作为一线脑脊液检测方法[51]。mNGS 有助于隐球菌的鉴定，与传统检测方法结合也可显著提高检出率[52]。mNGS 也能有效地检出传统检测方法不易发现的寄生虫[53]。对 204 名特发性脑膜炎、脑炎或脊髓炎患者进行急性感染性疾病的精确诊断研究，发现 mNGS 使感染诊断率提高了 22%。mNGS 与常规检测(培养、抗原检测和免疫原性检测)比较，阳性符合率为 80%，阴性符合率为 98%[10]。mNGS 可进一步分析耐药基因和毒力因子有助于临床医生合理有效地用药[11]，并能持续监测疾病进展和治疗效果从而帮助临床医生及

时地调整治疗方案[13]。mNGS 还被应用于 CNS 微生物群落探究,加强对人中枢神经系统的认识，有助于临床诊断和治疗 CNS 感染相关疾病[54]。mNGS 联合其他病原检测技术，有利于临床精准诊疗，可提升 CNS 感染的诊断率[10,55]。mNGS 还被应用于预测和监测新突发传染病疫情[56]。

4 mNGS 应用于 CNS 感染病原检测的局限性

mNGS 有利于 CNS 感染病原体的检出，但其广泛应用尚受诸多因素的限制。高宿主核酸占比严重影响病原的检出，现有去宿主核酸方法的效果还不够理想，更高效率的方法亟待开发。mNGS 结果易受检测样本、试剂和实验室环境中背景微生物的干扰，严格遵守检测流程的质量控制程序非常重要。此外，定期检测常见背景微生物菌群也有利于避免假阳性检出结果。病原参考数据库方面也存在不足之处：当前部分微生物基因组序列中仍存在空白和错误;针对CNS感染的专用病原数据库亟待开发。相较于 DNA 病原，mNGS 检测 RNA 病原的灵敏度尚存在一定的不足。预扩增方法可增加病原 RNA 的丰度，提升病原检出率。由于 CNS 感染性疾病的 mNGS 诊断尚缺乏标准的检测流程和规范的报告解读标准，未来该技术还需要不断地优化和完善。尽管 mNGS 在 CNS 感染性疾病诊疗中发挥了重要的作用，临床医生依然不能完全依赖于 mNGS 的病原检测结果，应结合患者病史和其他检测指标等信息筛选出主要致病病原以诊断病因[57]。当前，mNGS 检测的成本还比较高，限制了其在 CNS 感染病原检测的广泛应用。随着 mNGS 技术的成熟，其检测成本将有所下降。

5 总结和展望

mNGS 在 CNS 感染性疾病诊疗中已展现出了巨大的优势和潜力，但临床实践中仍面临诸多挑战。微生物学家和临床医生等应携手一同优化和完善 mNGS 技术，推动该技术临床应用的规范化。需要特别说明的是，应用 mNGS 技术不应完全摒弃传统检测方法，只有充分结合并发挥各种方案的优势才能更好地服务于 CNS 感染性疾病的快速精准诊疗。我们相信基于 mNGS 技术的 CNS 感染性疾病诊疗将成为临床微生物学的重要研究前沿之一。mNGS 技术将有力地推动 CNS 感染性疾病病原精准诊断并提升病患生存率。

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