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Hans汉斯

肾病综合征合并肺部感染宏基因组测序技术的诊断价值

陈　婷[1*]，付　甜[2*]，刘金彦[3#]

[1]济宁医学院临床医学院，山东　济宁
[2]济宁市第一人民医院呼吸科，山东　济宁
[3]济宁市第一人民医院肾内科，山东　济宁

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摘　要

肾病综合征(nephrotic syndrome, NS)是一种由机体免疫功能异常引起肾脏超微结构改变的肾脏疾病综合征。致病菌复杂的肺部感染(pulmonary infection, PI)是NS的常见并发症。宏基因组测序技术(metagenomics next-generation sequencing, mNGS)作为一种新兴的诊断技术，以其高敏感性的优势备受关注。本文针对mNGS对于NS合并PI的诊疗价值进行分析与探讨，为临床决策助一臂之力。

关键词

肾病综合征，肺部感染，宏基因组测序

Diagnostic Value of Metagenomics Next-Generation Sequencing in Nephrotic Syndrome Complicated with Pulmonary Infection

Ting Chen[1*], Tian Fu[2*], Jinyan Liu[3#]

[1]School of Clinical Medicine, Jining Medical University, Jining Shandong
[2]Department of Respiratory Medicine, Jining NO. 1 People's Hospital, Jining Shandong
[3]Department of Nephropathy, Jining NO. 1 People's Hospital, Jining Shandong

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[*]共同第一作者。
[#]通讯作者。

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Abstract

Nephrotic syndrome is a kind of kidney disease syndrome caused by abnormal immune function. It is found that complicated pulmonary infection with pathogenic bacteria is a common complication of Nephrotic syndrome. Metagenomics next-generation sequencing, as a new diagnostic technology, has attracted much attention because of its high sensitivity. In this paper, the diagnosis and treatment value of mNGS for Nephrotic syndrome complicated with PI is analyzed and discussed, which will help clinical decision.

Keywords

Nephrotic Syndrome, Pulmonary Infection, mNGS

1. 引言

NS 是因免疫功能异常所致肾脏滤过屏障受损，出现大量蛋白尿、低蛋白血症、水肿、高脂血症，目前激素联合免疫抑制剂、细胞毒药物以及靶向药物调节免疫是其主流治疗方案，其带来最主要的副作用是 PI，难以治愈，极易复发[1]。本文主要针对 mNGS 技术的临床应用展开分析与探讨，提高 NS 合并 PI 的诊疗效率。

2. NS 合并 PI 的发病机制与临床特点

2.1. NS 的概述

NS 是由于异常免疫复合物攻击肾脏致使肾间质发生炎症改变是 NS 的主要发病机制[2]。大量蛋白体外流失，存留于体内以及肝脏代偿性合成的白蛋白难以满足生理活动的需要，血浆胶体渗透压下降并向组织外渗，循环血容量减少，肾小球球旁器以及肾素－血管紧张素－醛固酮系统激活造成水钠潴留，加重全身组织水肿[3]。白蛋白含量过低致使肝脏合成功能亢进，脂蛋白的产生也随之增加，脂蛋白脂酶活性降低诱发代谢综合征[4]。目前肾穿刺活检是 NS 确定病理类型的金标准，其治疗原则以控制原发病为起点，以减缓肾功能恶化为最终目标[5]。泼尼松、强的松龙等糖皮质激素可抑制炎症因子的产生，减少趋化因子和前列腺素的表达[6]，激素联合环孢素、环磷酰胺，以利妥昔单抗为代表的生物制剂在临床中应用较为普遍[7] [8] [9] [10]。

2.2. NS 患者发生肺部感染风险明显增加

由于白蛋白的合成不足、体外流失以及机体消耗增加，以白蛋白为载体的营养物质未能运输至胞内，NS 患者往往处于营养不良的状态[11]。组织液大量留存与胸、腹腔等第三间隙为细菌提供了良好的培养基，致病性 B 细胞的异常激活打破免疫稳态，免疫球蛋白异常产生，流失过多，T 细胞介导的细胞免疫难以精准清除抗原，淋巴细胞比率降低，病原微生物在体内大量定植并向远处播散[12] [13]。免疫抑制药物虽然可以减少蛋白质从肾脏中流失，但大剂量的冲击疗法往往使大量胸腺细胞凋亡，巨噬细胞、NK

细胞和细胞毒性 T 淋巴细胞等多种免疫细胞数量减少，抗体的产生量锐减，免疫系统的防御、监测、清除能力不足，NS 患者对病原菌的易感性增加[14] [15]。严重感染加重肾组织缺血缺氧，氧化应激产生的自由基影响肾损伤后的修复，NS 向急性肾衰竭方向迈进[16]。

Li 等人通过对 1202 例原发性肾病综合征合并肺部感染住院患者感染菌群分析如下：在非重症感染组，肺部感染最常见的微生物是肺炎克雷伯菌；重症感染组以曲霉菌、诺卡氏菌、卡氏肺孢子虫病、结核杆菌和隐球菌为主的条件致病菌多见[17]。Wang 等人报道了肾综合征患者粪类圆线虫与克雷伯菌合并感染的案例：接受激素治疗的患者更容易出现幼虫增殖失控，过度感染综合征[18]。Cheng 等人发现诺卡氏菌属入血可引起长期接受糖皮质激素和免疫抑制剂治疗的 NS 患者发生肺部、浅表皮肤和皮下等多部位机会性感染[19]。胡瑞兰等人报道了一则 NS 患者有发热伴气短的临床表现，经支气管肺泡灌洗液培养检出肺孢子菌孢囊。经过治疗后，感染症状稍有缓解，但因患者高龄、一般情况差，再度感染鲍曼不动杆菌，最终出现呼吸衰竭而死亡[20]。念珠菌属通常情况下定植于口腔中，接受免疫抑制治疗的肾病综合征患者常因宿主防御机制受损更容易发生念珠菌入侵以及呼吸道广泛感染[21]。近些年来，创伤弧菌、隐球菌、放线菌、毛霉菌、新冠病毒多种病原体引起 NS 患者发生急性呼吸窘迫综合征多有报道[22] [23] [24] [25]。

3. mNGS 技术的应用现况与诊断效能评价

临床症状、胸部 CT、胸部 X 线可初步筛查并发现 PI。痰培养、咽拭子、鼻拭子、支气管肺泡灌洗、肺活检等病原学检测可对致病菌群进行鉴别与分离。传统病原微生物的提取方式虽然方便快捷，但因病原菌培养时间长，多数病原微生物不能在培养基上生长，显微镜涂片准确性低，漏诊率高，肺活检操作创伤大且临床应用多有局限性。以经验治疗为主体地位往往导致药物滥用，耐药性菌株的出现使患者错过最佳诊疗时机[26]。随着 NS 患者合并 PI 发病率不断上升，传统病原体检测技术在灵敏度、诊断效率方面难以满足临床医师的需求，mNGS 应运而生。

Yue 等人报道了第一例通过 mNGS 早期诊断为军团菌和侵袭性肺曲霉病(invasive pulmonary aspergillosis, IPA)并成功治疗的案例，mNGS 与传统诊断方法相比，mNGS 的灵敏度为 78.3%，特异性达 97.5%；由于霉菌中的核酸难以提取，mNGS 的灵敏度相对较低，军团菌感染、流感病毒感染以及免疫抑制是 IPA 感染的潜在危险因素[27]。mNGS 的方法学的应用能够从原始临床样本中直接提取病原微生物，揭示生物体内包括 RNA 病毒、DNA 病毒、细菌和真核生物的整个“感染体”，揭示了病原体丰度和基因表达的相关数据，科学高效的遏制肺炎相关传染病的大流行[28]。Wang 等人收集了 36 例被临床诊断为混合性 PI 的患者，通过常规检测手段与支气管肺泡灌洗液(broncho-alveolar lavage fluid, BALF) mNGS 检查回顾性分析，mNGS 结果示 31 例患者存在真菌感染，常规检测手段发现真菌感染的患者的仅 10 例，在免疫力低下的人群中，常有巨细胞病毒和肺孢子虫合并感染[29]。Feng 对 mNGS 和微生物培养在 PI 诊断中的有效性以及群落物种多样性进行评估：BALF 的 mNGS 对 PI 的诊断敏感性为 88.89%，特异性为 14.86%，阳性预测值为 21.16%，阴性预测值为 83.87%，排除罕见病原体后，mNGS 的敏感性降至 73.33%，特异性增至 41.71%，单纯 PI 组和免疫功能低下组的主要感染类型分别为细菌感染和混合感染[30]。

mNGS 相对于传统 PCR 的分子诊断来说后者需要引物以检测特定病原体，这一过程中存在遗漏罕见病原体或错误使用与被测病原体与引物不匹配情况从而导致检测失败，因此 PCR 更加适用于物种间致病菌的鉴定；mNGS 在诊断罕见或新的病原体方面具有更高的敏感性，但容易受到背景菌群的干扰，病原体分析的结果需临床医生结合患者临床表现和常规实验室检查如传统培养、G 试验、GM 试验、抗体试验、血清抗原试验等综合分析[31]。肺组织活检联合 mNGS 的检测效果相比，mNGS 结合涂片分析可作为侵袭性真菌感染的诊断工具，肺组织在研磨机中均质，研磨处理可能会影响接合菌(如根霉菌和毛霉菌)的分离，后续进行涂片和培养的结果往往存在偏差[32]；肺活检的 mNGS 产生的读数部分来自口咽或皮

肤中的细菌，是否为致病菌群需要结合临床表现和相关实验室检查结果综合考虑[33]。在抗生素敏感性和耐药性基因检测方面，传统方法检测病原体耗时长，而且忽略了不可培养的细菌，mNGS 的抗生素敏感性试验(antimicrobial susceptibility tests, AST)显示了含有耐药基因的细菌的耐药性，但一些多重耐药细菌的耐药基因并未检测到[34]。

4. NS 合并 PI，mNGS 的利弊分析

4.1. mNGS 的优势与诊断价值

mNGS 是一种高通量测序方法[35]，从临床原始数据中筛选病原体，生成每个样本的综合报告，与生物信息相结合，普遍用于全基因组测序、重测序、转录组测序、RNA 测序、DNA 测序、蛋白质相互作用和 DNA 甲基化分析等领域[36]；将其与特征序列数据库进行比较，展示了多微生物生态系统的原貌、扩展微生物种群的特征与亚型以及不同基础疾病患者呼吸道微生物和优势菌群的分布[37]；潜在的病原体可以根据 DNA 或 RNA 的相对丰度来识别，检测范围更广泛[38]；抗生素的应用不影响检测结果，阳性结果可作为肺部感染药物治疗的靶向处方，结果读数的动态变化是评价患者预后的有效手段[39]，样本需求量少，检验周期短[40]，可用于后续筛选读数、组装重叠、细菌种类的相对丰度及抗药性基因的分析、毒力因子的鉴定以及诊断结果质量检查[40]；对罕见的和难以培养的病原菌有较高的阳性检出率，[41]在疫情监测与防控方面发挥中流砥柱的作用[42]。因此在经济条件允许的情况下，mNGS 可选择作为常规辅助检测方法甚至可作为肺部感染的主流方式，这对 NS 合并 PI 的早诊断、早治疗具有重要意义。

4.2. mNGS 的劣势与对策思考

目前 mNGS 的临床应用更倾向于检测脑脊液和血液等背景菌群较为简单的样本，在尿液、粪便、痰液、支气管肺泡灌洗液等背景菌群复杂的样本中，mNGS 的实施仍处于验证阶段[43]。在 NS 患者体内，几乎所有的细菌或真菌都可以被认为是肺部感染的潜在病原体，所以很难精准的确定致病菌[37]；mNGS 受背景菌群的影响，可能出现假阳性结果；在 NS 合并 PI 等免疫功能低下的人群可能面临多重感染，由于缺乏公认的解释测序结果的标准，mNGS 对病原微生物的诊断极易存在偏差[44]；由于部分 RNA 病毒发生自动降解，mNGS 会出现假阴性的检出结果[45]；对寄生虫诊断的准确性取决于寄生虫在样本中的数量和分布，技术人员对寄生虫形态特征的经验性识别等主观因素使寄生虫的诊断无法得到不偏不倚的结果[46]。在耐药性分析方面，短读长度基本上不超过 300 bp，只有少数特定序列可以检测到，虽然有研究者提出并验证了数据整合和组装可以帮助获得耐药结果，但并没有被广泛应用于临床实验室进行复杂的数据处理，低丰度的检测是 mNGS 临床耐药性分析的核心挑战[47] [48]；外排泵编码基因、外膜蛋白和 β-内酰胺酶耐药基因等内源性耐药基因的出现导致多种菌群耐药性的出现[26]，mNGS 具有与多个已知耐药位点匹配的潜力，但没有包含所有抗药性基因或与抗药性相关的基因点突变的数据库；由于缺乏对基因突变引起的病原体耐药性的理解和新耐药机制的出现，mNGS 预测耐药性较为困难[49]。在临床使用方面，与培养检测相比，mNGS 价格昂贵，且尚未纳入医疗保险，这在一定程度上限制了 mNGS 在临床实践中的推广[35]。

一个准确、全面的 mNGS 数据库需要专业人员进行精确的分类鉴别，以免出现假阴性或假阳性结果；一个真正的阳性结果的序列应该在整个基因组中随机分布，而不是集中在一个狭窄的范围内，编制一份完整的保留匹配的读数的病原体清单，对 mNGS 数据的解释应结合常规实验室诊断方法的结果[49]；Hua 等人对 159 例患者开展进行肺炎病原体鉴定的临床前瞻性研究，结果显示 mNGS 比标准方法(包括培养、染色和靶向 PCR)鉴定出更多的微生物(117:72)，检测出标准方法在所有病例中遗漏的 17 种病原体，也有助于保证 19 例(11.9%)的抗生素降级[50]；mNGS 应有一个明确的检测阈值，临床医师深入了解病原体的

特性，结合病情变化谨慎解读常规方法的结果不一致的 mNGS 结果[43]。病原体及耐药相关基因数据库规模有待扩大，基于这些数据库的 mNGS 结果解释的准确性有待提高。

5. 总结

接受免疫抑制治疗的 NS 患者合并 PI 的诊治对于临床医师来说是一个严峻的挑战。PI 的快速诊断对于前期治疗和后期预后大有裨益。重建免疫稳态是接受免疫抑制治疗的 NS 患者远离呼吸道感染的关键一环。病原体的早期检测可以优化抗生素的管理，缩短住院时间。无论使用何种药物，复发都不能完全避免，治疗后随访可以大幅度提高生存率。未来的 mNGS 技术将通过不断改革创新弥补不足，逐渐成为临床微生物学诊断的革命性技术，为 NS 合并 PI 的诊断提供新的视角，引导临床医生从经验诊断向基因组诊断过渡，实现未病先防，提高生存率。

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