

# 急性冠脉综合征患者血浆外泌体 NEAT1, miR-204 和 MMP-9 的表达水平及临床意义

丁 锦<sup>a</sup>, 贯 芳<sup>b</sup>, 梁小涵<sup>c</sup>, 陈 旭<sup>a</sup>

(首都医科大学附属北京世纪坛医院 a. 急诊科; b. 消化内科; c. 胃肠肝胆肿瘤外科, 北京 100038)

**摘要:** 目的 检验急性冠脉综合征 (acute coronary syndrome, ACS) 患者血浆外泌体富含核富集的转录物 1 (Nucleolar-enriched autosomal transcript, NEAT1), 微小核糖核酸 (microRNA, miR)-204 和基质金属蛋白酶 (metalloproteinase, MMP)-9 的表达水平, 并研究其对 ACS 的诊断价值, 分析其与冠脉病变严重程度的相关性。方法 选取 2020 年 5 月~2021 年 5 月于首都医科大学附属北京世纪坛医院诊断为 ACS 的 120 例患者作为实验组, 其中急性心肌梗死 (acute myocardial infarction, AMI) 患者 72 例, 不稳定心绞痛 (unstable angina, UA) 患者 48 例; 另选取行冠脉造影检查无明显狭窄的 108 例体检者作为 Control 组。提取所有研究对象的血浆外泌体, 并采用透射电镜和 Western blot 鉴定该外泌体。应用试剂盒提取血浆外泌体总 RNA, 采用 qRT-PCR 法检测外泌体 NEAT1 和 miR-204 的表达水平, 采用 Western blot 检测外泌体 MMP-9 蛋白表达水平。应用 Pearson 相关分析明确外泌体 NEAT1, miR-204 和 MMP-9 水平与病变严重程度评分 (Gensini 评分) 之间的相关性。Logistic 回归模型判断外泌体 NEAT1, miR-204 和 MMP-9 是否可作为诊断 ACS 的独立危险因素。绘制受试者工作特征 (ROC) 曲线, 根据曲线下面积 (AUC) 分析三者表达水平对 ACS 病变严重程度的预测价值。结果 透射电镜观察到, 血浆外泌体呈椭圆形双层膜囊泡结构, Western blot 结果显示, CD63 和 CD81 蛋白在外泌体中呈高表达。Control 组 NEAT1, miR-204 和 MMP-9 表达水平分别为  $0.62 \pm 0.08$ ,  $1.02 \pm 0.20$  和  $0.97 \pm 0.15$ , UA 组患者 NEAT1, miR-204 表达水平分别为  $0.65 \pm 0.11$ ,  $1.05 \pm 0.18$ , 与 Control 组比较差异无统计学意义 ( $t=2.790$ ,  $3.225$ ,  $P>0.05$ ), 而 MMP-9 表达水平 ( $1.15 \pm 0.20$ ) 较 Control 组明显升高, 差异有统计学意义 ( $t=13.682$ ,  $P<0.05$ ); AMI 组患者血浆外泌体 NEAT1, miR-204 和 MMP-9 表达水平分别为  $0.98 \pm 0.15$ ,  $1.22 \pm 0.23$  和  $1.37 \pm 0.25$ , 较 Control 组明显升高, 差异均有统计学意义 ( $t=12.112$ ,  $9.885$ ,  $21.530$ , 均  $P<0.05$ )。Pearson 相关性分析显示, AMI 患者外泌体 NEAT1, MMP-9 与 Gensini 评分呈中度正相关, miR-204 与 Gensini 评分呈弱正相关, 差异有统计学意义 ( $r=0.179 \sim 0.548$ , 均  $P<0.05$ )。UA 患者外泌体 NEAT1 与 Gensini 评分呈弱正相关 ( $r=0.207$ ,  $P=0.032$ ), MMP-9 与 Gensini 评分呈中度正相关 ( $r=0.574$ ,  $P<0.05$ ), 但 miR-204 与 Gensini 评分无相关性 ( $r=0.108$ ,  $P=0.465$ )。Logistic 回归分析结果显示, 外泌体 NEAT1, miR-204 和 MMP-9 均可作为 AMI 预测的独立危险因素, 但外泌体 miR-204 不可作为预测 UA 的独立危险因素。ROC 结果显示, AMI 组外泌体 NEAT1, miR-204 和 MMP-9 水平及联合检测对应 AUC 分别为 0.821, 0.702, 0.750 和 0.905, UA 组外泌体 NEAT1, miR-204 和 MMP-9 水平及联合检测对应 AUC 分别为 0.776, 0.682, 0.718 和 0.883, 三者对 AMI 的诊断价值均高于 UA, 且联合检测具有更高诊断价值。结论 血浆外泌体 NEAT1, miR-204 和 MMP-9 具有预测冠状动脉病变严重程度的潜力, 并且三者联合检测对 AMI 具有较高诊断价值, 可作为辅助诊断 AMI 的潜在标志物。关键词: 血浆外泌体; 富含核富集的转录物 1 (NEAT1); 微小核糖核酸-204 (miR-204); 基质金属蛋白酶-9 (MMP-9); 急性冠脉综合征

中图分类号: R541.4; R392.11 文献标识码: A 文章编号: 1671-7414 (2023) 01-059-07

doi:10.3969/j.issn.1671-7414.2023.01.012

## Expression Levels and Clinical Significance of Plasma Exosomal NEAT1, miR-204 and MMP-9 in Patients with Acute Coronary Syndrome

DING Jin<sup>a</sup>, GUAN Fang<sup>b</sup>, LIANG Xiao-han<sup>c</sup>, CHEN Xu<sup>a</sup>

(a. Department of Emergency; b. Department of Gastroenterology; c. Department of Gastrointestinal and Hepatobiliary Tumor Surgery, Beijing Shijitan Hospital Affiliated to Capital Medical University, Beijing 100038, China)

**Abstract: Objective** To investigate the expression level of plasma exosome nucleolar-enriched autosomal transcript (NEAT1), microRNA-204 (miR-204) and metalloproteinase (MMP)-9 in patients with acute coronary syndrome (ACS), and study its diagnostic value for ACS and its correlation with the severity of coronary lesions. **Methods** 120 patients with

作者简介: 丁锦 (1995-), 女, 本科, 主治医师, 研究方向: 急性心肌梗死, E-mail: dingjin@bjsjth.cn。

通讯作者: 陈旭 (1980-), 女, 本科, 副主任医师, 研究方向: 急性心肌梗死。

ACS diagnosed in the Department of Cardiology of Beijing Shijitan Hospital Affiliated to Capital Medical University from May 2020 to May 2021 were selected as the experimental group, including 72 patients with acute myocardial infarction (AMI) and 48 patients with unstable angina pectoris (UA). In addition, 108 patients hospitalized in the department in the same period and found no obvious coronary stenosis by coronary angiography were selected as the control group. The plasma exosomes of all subjects were extracted and identified by transmission electron microscopy and Western blot. The total RNA of the above exosomes was extracted by the kit, the expression levels of exosomal NEAT1 and miR-204 were detected by qRT-PCR, and the protein expression levels of exosomal MMP-9 were detected by Western blot. Pearson correlation analysis was used to clarify the correlation between exosomal NEAT1, miR-204, MMP-9 levels and lesion severity score (Gensini score). Logistic regression model to determine whether exosomal NEAT1, miR-204 and MMP-9 could be used as an independent risk factor for the diagnosis of ACS. Draw the receiver operating characteristic (ROC) curve, and analyze the predictive value of the three expression levels on the severity of ACS lesions according to the area under the curve AUC. **Results** Transmission electron microscope observed that the plasma exosomes had an oval double-membrane vesicle structure. Western blot results showed that CD63 and CD81 proteins were significantly expressed in exosomes. The expressions of NEAT1, miR-204 and MMP-9 in the Control group were  $0.62 \pm 0.08$ ,  $1.02 \pm 0.20$  and  $0.97 \pm 0.15$ , respectively. The expressions of NEAT1 and miR-204 in the UA group were  $0.65 \pm 0.11$  and  $1.05 \pm 0.18$ , respectively, with no significant difference compared with the Control group ( $t=2.790$ ,  $3.225$ ,  $P > 0.05$ ), while the expression level of MMP-9 ( $1.15 \pm 0.20$ ) was significantly higher than that in the Control group, the difference was statistically significant ( $t=13.682$ ,  $P < 0.05$ ). The expressions level of plasma exosomal NEAT1, miR-204 and MMP-9 in the AMI group were  $0.98 \pm 0.15$ ,  $1.22 \pm 0.23$  and  $1.37 \pm 0.25$ , respectively, which were significantly higher than those in the Control group, and the differences were statistically significant ( $t=9.885 \sim 21.530$ , all  $P < 0.05$ ). Pearson correlation analysis showed that exosomal NEAT1, MMP-9 and Gensini scores were moderately positively correlated in AMI patients, and miR-204 was weakly positively correlated with Gensini scores, and the differences were statistically significant ( $r=0.179 \sim 0.548$ , all  $P < 0.05$ ), exosomal NEAT1 in UA patients was weakly positively correlated with Gensini score ( $r=0.207$ ,  $P=0.032$ ), and MMP-9 was moderately positively correlated with Gensini score ( $r=0.574$ ,  $P < 0.05$ ), but there was no significant difference between miR-204 and Gensini score ( $r=0.108$ ,  $P=0.465$ ). Logistic regression analysis showed that exosomal NEAT1, miR-204 and MMP-9 could be used as independent risk factors for AMI, however, exosomal miR-204 could not be used as an independent risk factor for UA prediction. The ROC results showed that the levels of exosomal NEAT1, miR-204 and MMP-9 in the AMI group and the corresponding AUC of the combined detection were 0.821, 0.702, 0.750 and 0.905, respectively. The levels of exosomal NEAT1, miR-204 and MMP-9 in the UA group and the corresponding AUCs of the combined detection were 0.776, 0.682, 0.718 and 0.883, respectively. The diagnostic value of the three for AMI was higher than that of UA, and the combined detection had a higher diagnostic value. **Conclusion** Plasma exosomal NEAT1, miR-204 and MMP-9 had the potential to predict the severity of coronary artery lesions, and the combined detection of the three has high diagnostic value for AMI, and could be used as potential markers for auxiliary diagnosis of AMI.

**Keywords:** plasma exosomes; nucleolar-enriched autosomal transcript 1 (NEAT1); micro RNA (miR)-204; metalloproteinase (MMP)-9; acute coronary syndrome

目前,临床诊断急性冠脉综合征(acute coronary syndrome, ACS)时除了根据患者的临床症状外,主要依赖于冠脉造影,该方法费用昂贵,且对于造影剂过敏、甲状腺功能亢进症以及肾功能不全等特殊患者,具有高风险性,在临床诊断时无法全面推广<sup>[1-2]</sup>。因此,寻找与ACS发生发展相关的新型生物标志物,分析其表达水平对ACS的诊断价值,具有重要的临床意义。有研究报道,外泌体作为自身载体,广泛参与了机体免疫应答、细胞分化、肿瘤生长等各种生物过程中<sup>[3]</sup>。富含核富集的转录物1(nucleolar-enriched autosomal transcript 1, NEAT1)是11号染色体转录的长链非编码RNA,已有研究证明它可以促进心肌缺血再灌注损伤,

在心血管疾病发生发展中发挥着重要作用<sup>[4]</sup>。微小核糖核酸(micro RNA, miR)-204是一种抑癌剂,其高表达可以抑制糖尿病小鼠心肌细胞发生自噬,从而缓解缺氧/复氧损伤,在心血管疾病治疗中具有潜在的临床应用价值<sup>[5]</sup>。基质金属蛋白酶(metalloproteinase, MMP)-9作为家族中的重要成员,病理状态下MMP-9被激活,通过削减斑块参与多种心血管疾病的发生发展<sup>[6]</sup>。基于前期研究发现,NEAT1, miR-204和MMP-9与心血管疾病的发生发展密切相关,可推测NEAT1, miR-204和MMP-9可能参与ACS的病理过程。故本研究检测了急性心肌梗死(acute myocardial infarction, AMI)和不稳定心绞痛(unstable angina pectoris, UA)患

者血浆外泌体中 NEAT1, miR-204 和 MMP-9 的表达情况,并分析其表达水平对 ACS 的诊断价值及其与冠脉病变严重程度的相关性,以期对 ACS 的后期诊断提供实验依据。

## 1 材料与方法

**1.1 研究对象** 选取 2020 年 5 月 ~ 2021 年 5 月于首都医科大学附属北京世纪坛医院内科诊断为 ACS 的 120 例患者作为实验组,其中急性心肌梗死患者 72 例,男性 42 例,女性 30 例,平均年龄  $70.86 \pm 5.18$  岁,收缩压  $133.54 \pm 13.15$  mmHg,舒张压  $79.13 \pm 8.55$  mmHg,身体质量指数 (body mass index, BMI)  $25.70 \pm 2.16$  kg/cm<sup>2</sup>,总胆固醇 (TC)  $4.18 \pm 0.30$  mmol/L,三酰甘油 (TG)  $1.50 \pm 0.28$  mmol/L,高密度脂蛋白-胆固醇 (HDL-C)  $1.37 \pm 0.29$  mmol/L,低密度脂蛋白-胆固醇 (LDL-C)  $2.88 \pm 0.57$  mmol/L;不稳定心绞痛患者 48 例,男性 29 例,女性 19 例,平均年龄  $70.27 \pm 5.89$  岁,收缩压  $131.27 \pm 14.77$  mmHg,舒张压  $78.92 \pm 7.68$  mmHg, BMI  $25.08 \pm 2.50$  kg/cm<sup>2</sup>, TC  $4.11 \pm 0.44$  mmol/L, TG  $1.42 \pm 0.33$  mmol/L, HDL-C  $1.33 \pm 0.35$  mmol/L, LDL-C  $2.80 \pm 0.65$  mmol/L。另选取同期我院体检中心行冠脉造影者且无明显狭窄的 108 例体检者作为 Control 组,男性 60 例,女性 48 例,平均年龄  $71.16 \pm 5.42$  岁,收缩压  $129.16 \pm 13.42$  mmHg,舒张压  $76.60 \pm 7.28$  mmHg, BMI  $25.51 \pm 1.98$  kg/cm<sup>2</sup>, TC  $4.07 \pm 0.39$  mmol/L, TG  $1.47 \pm 0.19$  mmol/L, HDL-C  $1.31 \pm 0.30$  mmol/L, LDL-C  $2.67 \pm 0.68$  mmol/L,三组间上述一般资料相比较,差异均无统计学意义 (均  $P > 0.05$ ),具有可比性。

**纳入标准:** ①根据最新发布的急性冠脉综合征急诊快速诊治指南以及“全球心肌梗死定义”标准,分别诊断为 AMI 和 UA 的患者;②具有缺血性胸痛症状;③冠状动脉造影显示至少存在 1 支狭窄超过 75% 的冠状动脉;④本研究经本院伦理委员会审核批准后进行 (L20200102),所有研究对象均知情并同意。**排除标准:** ①既往接受过经皮冠状动脉介入治疗者;②患有其他类型心脏疾病者;③并发其他恶性肿瘤者;④患有自身免疫病者。

表 1

qRT-PCR 引物序列

名称	正向引物	反向引物
NEAT1	5'-CTTCCTCCTTTAACTTATCCAT-3'	5'-CTCTCTCCACCATTACCAACAAT-3'
GAPDH	5'-AGAAGGCTGCTCATTTG-3'	5'-AGGTTCATCCACAGTCTTC-3'
miR-204	5'-GTGAGCGTCCGACGT-3'	5'-GCCGGTACGTTATCAGTGT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

**1.3.4 Western blot 检测血浆外泌体中 MMP-9 蛋白表达:** 根据外泌体蛋白提取试剂盒说明书提取血浆

**1.2 仪器与试剂** exoRNeasy Kit 试剂盒 (上海力敏实业有限公司); RNA 提取试剂盒,逆转录试剂盒 (北京 Solarbio 有限公司); miRNAs 引物 (上海生工生物工程公司合成); MMP-9 抗体 (上海碧云天生物科技有限公司); 离心机 (赛默飞世尔科技有限公司); 荧光定量 PCR 仪 (美国 Bio-Rad 公司)。

## 1.3 方法

**1.3.1 血液样品预处理:** 入院后先对所有研究对象的年龄、性别、身高、体重、舒张压、收缩压等一般资料进行记录,采所有研究对象的空腹外周静脉血,加入提前装有 EDTA-Na<sub>2</sub> 的抗凝管,3 000 r/min,4 ℃离心 10 min 后,将上层淡黄色血浆转移至无 RNA 酶离心管中,于 -80 ℃冰箱保存。

**1.3.2 血浆外泌体提取及鉴定:** 从 -80 ℃冰箱取出冻存血浆后,常温解冻,3 000 r/min,4 ℃离心 10 min 后,转移上层液体至新的无 RNA 酶离心管中,严格按照 exoRNeasy Kit 试剂盒说明书提取血浆外泌体,取 5 μl 外泌体重悬液滴于 150 目铜网上,室温放置 5 min,吸除多余液体;在铜网上滴加 5 μl 浓度为 7 mol/ml 的磷钨酸溶液,染色 5 min,滤纸吸去多余液体,静置晾干,在透射电子显微镜下观察外泌体形态,并用 Western blot 实验测定外泌体特征性蛋白 CD63 和 CD81。

**1.3.3 qRT-PCR 检测外泌体 NEAT1 和 miR-204 表达:** 根据 RNA 提取试剂盒操作说明提取血浆外泌体总 RNA,并取 2 μl 检测其纯度,  $A_{260nm}/A_{280nm}$  在 1.8 ~ 2.0 之间即为纯度较高,可进行后续实验。采用反转录试剂盒第一链 cDNA,反转录体系 20 μl,反应条件: 37 ℃ 15 min (反转录反应), 85 ℃ 5s (反转录酶的失活反应),结束后 cDNA 置 4 ℃保存。用上述合成的第一链 cDNA 作为模板,应用 SYBR Green Mix 进行实时 PCR 分析,反应体系为 25 μl,实时 PCR 条件: 94 ℃ 5 min, 94 ℃ 30 s, 60 ℃ 30 s 循环 35 次, 72 ℃ 10 min, 4 ℃保存备用。分别以 GAPDH 和 U6 RNA 作为内参基因,根据 RT-PCR 反应结果中的 Ct 值,采用相对定量的方法计算外泌体中 NEAT1 和 miR-204 的相对表达量。引物序列见表 1。

外泌体总蛋白,并将样品的蛋白浓度调至一致后加入 loading buffer,混匀后煮沸 10 min,冷却至室温



后离心,在提前配置好的胶孔中加入蛋白样品后,上层浓缩胶在80 V恒压进行,待跑至分离胶时调整为120 V恒压,电泳结束后,110 mA恒流条件下转NC膜2 h,加入100 ml的0.05 g/ml脱脂奶粉室温摇床封闭2 h,PBST洗膜、加入一抗4 ℃孵育过夜,回收一抗,PBST洗膜后加入二抗,室温孵育2 h,PBST漂洗3次后采用化学发光法进行显色,凝胶成像仪观察拍照,并用Image-J软件分析各组蛋白相对表达。

**1.3.5 血浆外泌体 NEAT1, miR-204, MMP-9 与 Gensini 评分之间的相关性:**冠状动脉病变严重程度评分采用 Gensini 评分系统:对每支冠状动脉病变狭窄程度进行定量评定:狭窄直径<25%计1分,25%≤狭窄直径<50%计2分,50%≤狭窄直径<75%计4分,75%≤狭窄直径<90%计8分,90%≤狭窄直径<99%计16分,≥99%计32分。根据不同冠脉分支将以上得分乘以相应系数,左主干病变:得分×5;左前降支病变:近端×2.5,中段×1.5;对角支病变:D1×1,D2×0.5;左回旋支病变:近端×2.5,远端×1;后降支×1;后侧支×0.5;右冠状动脉病变:近、中、远和后降支均×1。各病变支得分总和即为患者的冠状动脉病变狭窄程度总积分。

**1.3.6 Logistic 回归分析:**将外泌体 NEAT1, miR-204 和 MMP-9 作为变量进行多因素 logistic 回归分

析,判断三者是否可作为诊断 ACS 的独立危险因素。**1.3.7 绘制 ROC 曲线:**根据测量所得血浆外泌体 NEAT1, miR-204 和 MMP-9 表达水平绘制 ROC 曲线,并计算 AUC,诊断阈值、敏感度和特异度,进而来判断 NEAT1, miR-204 和 MMP-9 表达水平对 ACS 病变严重程度的预测价值。

**1.4 统计学分析** 应用 SPSS22.0 分析数据,计数资料以  $n(\%)$  表示,采用  $\chi^2$  检验;服从正态分布的计量资料以均数±标准差( $\bar{x} \pm s$ )表示,多组间差异比较采用单因素方差分析,两两比较采用独立样本  $t$  检验,相关性分析用 Pearson 法,以  $P < 0.05$  为差异有统计学意义。

## 2 结果

**2.1 血浆外泌体鉴定** 透射电镜观察到,血浆外泌体呈椭圆形双层膜囊泡结构,Western blot 结果显示,外泌体标志蛋白 CD63 和 CD81 在外泌体中呈明显表达,说明外泌体提取成功。

**2.2 血浆外泌体 NEAT1, miR-204 和 MMP-9 表达水平比较** 见表2。UA 组患者与 Control 组相比,NEAT1, miR-204 表达水平差异无统计学意义( $t=2.790, 3.225, P > 0.05$ ),而 MMP-9 表达升高,差异有统计学意义( $t=13.682, P < 0.05$ );与 Control 组相比,AMI 组患者血浆外泌体 NEAT1, miR-204 和 MMP-9 表达均上升,差异有统计学意义( $t=15.112, 9.885, 21.530$ , 均  $P < 0.05$ )。

表2 三组患者血浆外泌体 NEAT1, miR-204 和 MMP-9 表达水平比较( $\bar{x} \pm s$ )

项目	Control ( $n=108$ )	AMI 组 ( $n=72$ )	UA 组 ( $n=48$ )	$F$	$P$
NEAT1	0.62±0.08	0.98±0.15	0.65±0.11	52.538	<0.001
miR-204	1.02±0.20	1.22±0.23	1.05±0.18	75.260	<0.001
MMP-9	0.97±0.15	1.37±0.25	1.15±0.20	68.053	<0.001

**2.3 血浆外泌体 NEAT1, miR-204, MMP-9 与 Gensini 评分之间的相关性** Pearson 相关性分析显示,AMI 患者外泌体 NEAT1, MMP-9 与 Gensini 评分呈中度正相关( $r=0.446, 0.548$ );外泌体 miR-204 与 Gensini 评分呈弱正相关( $r=0.179$ ),差异均有统计学意义(均  $P < 0.05$ );UA 患者外泌体 NEAT1 与 Gensini 评分呈弱正相关( $r=0.207$ ),MMP-9 与 Gensini 评分呈中度正相关( $r=0.574$ ),差异亦有统计学意义( $P < 0.05$ ),但 miR-204 与

Gensini 评分无明显相关性( $r=0.108$ ),差异无统计学意义( $P > 0.05$ )。

**2.4 血浆外泌体 NEAT1/miR-204/MMP-9 对 AMI 和 UA 独立危险因素判断** 见表3、表4。Logistic 回归分析结果显示,外泌体 NEAT1, miR-204 和 MMP-9 均可作为 AMI 预测的独立危险因素( $P < 0.05$ ),但外泌体 miR-204 对 UA 的预测,差异无统计学意义( $P > 0.05$ ),不可作为独立危险因素。

表3 AMI 患者 Logistic 回归分析

类别	回归系数	标准误	Wald	OR	95%CI	$P$ 值
NEAT1	5.469	1.177	21.592	237.232	23.623 ~ 2 382.359	0.000
miR-204	5.260	1.252	17.648	192.480	16.542 ~ 2 239.652	0.000
MMP-9	3.118	0.990	9.918	22.603	3.246 ~ 157.369	0.002

表4 UA 患者 Logistic 回归分析

类别	回归系数	标准误	Wald	OR	95%CI	P 值
NEAT1	3.945	1.021	14.929	51.691	6.987 ~ 382.436	0.000
miR-204	1.194	1.372	0.758	3.302	0.224 ~ 48.591	0.384
MMP-9	2.603	1.013	6.604	13.506	1.855 ~ 98.354	0.010

2.5 血浆外泌体 NEAT1, miR-204, MMP-9 对 AMI 和 UA 诊断价值比较 见图 1。ROC 曲线结果显示, AMI 患者外泌体 NEAT1, miR-204 和 MMP-9 水平对应 AUC 分别为 0.821, 0.702 和 0.750, 联合检测 AUC 为 0.905; UA 患者外泌体 NEAT1,

miR-204 和 MMP-9 水平对应 AUC 分别为 0.776, 0.682 和 0.718, 联合检测 AUC 为 0.883, 差异均有统计学意义 (均  $P < 0.05$ ), 三者对 AMI 的诊断价值均高于 UA 患者, 但联合检测具有更高的诊断价值。

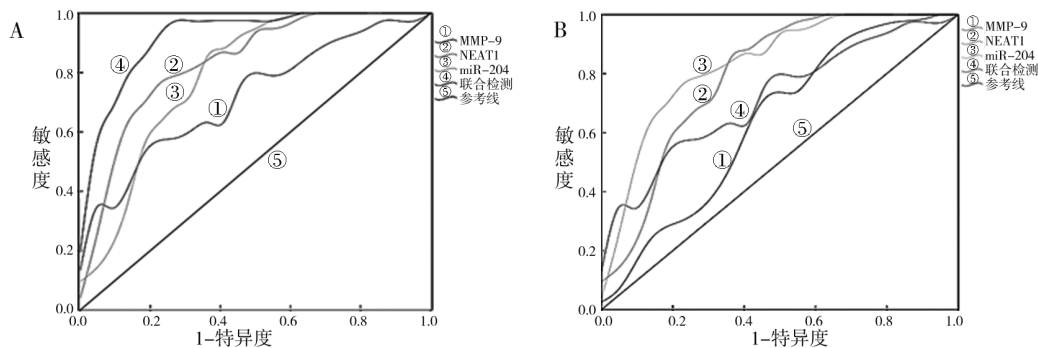


图1 外泌体 NEAT1, miR-204, MMP-9 及联合诊断对 AMI (A) 和 UA (B) 患者的预测价值比较

### 3 讨论

ACS 是心血管疾病中最严重的一大类疾病, 该类病人冠状动脉内血栓有分层结构, 容易引起相应部位动脉完全或部分闭塞, 可使冠脉痉挛, 造成心肌缺血、坏死, 继而进展为心衰, 严重威胁患者的生命健康<sup>[7]</sup>。因此, 寻找具有诊断价值的靶向因子来提高心肌梗死患者的生存率, 改善其不良预后具有十分重要的临床意义。近年来已有大量研究证实, 外泌体是低免疫原性的, 其能够穿过血脑屏障为受体细胞提供蛋白质、RNA 及 miRNA 等, 在心血管疾病的发生进程中起到一定的调节作用<sup>[8]</sup>。

MA 等<sup>[9]</sup>探讨了 NEAT1 对糖尿病大鼠离体和体内心肌缺血再灌注损伤的影响, 结果表明, NEAT1 过表达促进了乳酸脱氢酶的产生, 抑制了超氧化物歧化酶的含量和心肌细胞的活力, 并且通过促进心肌细胞凋亡和自噬, 加重糖尿病大鼠心肌缺血再灌注损伤。WANG 等<sup>[10]</sup>探讨了 NEAT1 在动脉粥样硬化进展中的生物学作用, 结果表明, 阻断 NEAT1 可以调节人类巨噬细胞 THP-1 细胞中的 miR-342-3p 表达, 进而抑制机体炎症反应, 可能为治疗动脉粥样硬化性心血管疾病的潜在生物标志物。LUO 等<sup>[11]</sup>探讨了 NEAT1 对心肌缺血再灌注损伤是否具有调节作用, 结果表明, NEAT1 在心肌缺血再灌注损伤小鼠中表达升高, 可能是通过调节 miR-495-3p 和有些分裂原激活蛋白酶 6 (MAPK6)

发挥作用。上述文献均证实 NEAT1 在调节心血管疾病中起关键作用, 本研究发现, AMI 患者 NEAT1 表达显著上升, 而 UA 患者 NEAT1 虽上升, 但差异无统计学意义, 且 NEAT1 表达与 Gensini 评分呈正相关, 可以预测冠脉病变严重程度。

miR-204 属于单链非编码 RNA, 已有研究证实 NEAT1 通过下调 miR-204 表达促进肿瘤细胞上皮间质转化, 且对心血管疾病具有多种保护作用<sup>[12-13]</sup>。DU 等<sup>[14]</sup>探讨了 miR-204 对阿霉素诱导的心肌损伤的影响, 结果表明, miR-204 通过抑制高迁移率族蛋白 1 (HMGB1) 来保护免受阿霉素诱导的心脏损伤, 增加 miR-204 的表达可能是阿霉素诱导的心脏损伤患者的新治疗选择。吴静等<sup>[15]</sup>研究了血清 miR-204 水平对经皮冠状动脉介入治疗 (PCI) 术后急性冠脉综合征 (ACS) 患者预后的评估价值, 结果表明, ACS 患者血清 miR-204 水平显著降低, 且其水平与介入治疗预后密切相关, 具有重要的临床价值。LUAN 等<sup>[16]</sup>探索了 miR-204 在体外缺氧/复氧 (H/R) 诱导的心肌细胞损伤中的作用, 结果表明, miR-204 在 H/R 处理后显著下调, 转染 miR-204 模拟物减弱了 H/R 诱导的受损细胞活力并增加了细胞凋亡率。本研究发现, AMI 患者 miR-204 表达显著上升, 且其表达水平与 Gensini 评分呈弱正相关, UA 患者 miR-204 略有上升, 但差异无统计学意义, 且其表达与 Gensini 评分无明显相关性, 对 AMI 具

有一定的预测价值。

MMP-9 作为基质金属蛋白,其表达水平升高则会导致纤维蛋白降解变薄,是斑块不稳定的重要诱因<sup>[17]</sup>。NANDI 等<sup>[18]</sup>探讨了 MMP-9 在慢性心力衰竭中的心脏保护作用,结果表明,心肌梗死后 MMP-9 的增加加剧了缺血诱导的慢性心力衰竭,抑制 MMP-9 表达在病理性心脏重塑中具有重要意义。陈俊等<sup>[19]</sup>探讨了 MMP-9 与心房颤动的关系及对持续性心房颤动患者复律治疗后复发的预测价值,结果表明, MMP-9 在心房颤动患者中表达升高,可能是心房颤动患者心房结构重构的主要因素之一,其可以影响胶原代谢、促进心肌纤维化、参与心脏结构重构,在房颤的发生和维持中发挥作用。此外,有研究证实 MMP-9 是 miR-204 的直接靶向因子,miR-204 通过抑制 MMP-9 表达来影响细胞侵袭<sup>[20]</sup>。本研究发现,AMI 和 UA 患者 MMP-9 表达均显著上升,且其表达水平与 Gensini 评分呈中度正相关,对冠脉病变严重程度具有较高的预测价值。

综上所述,血浆外泌体 NEAT1, miR-204 和 MMP-9 具有预测冠状动脉病变严重程度的潜力,并且三者联合检测对 AMI 具有较高诊断价值,可作为辅助诊断 AMI 的潜在标志物。但本实验的样本数量较少,后期可以通过纳入更多样本量来进一步验证,还可结合细胞实验进一步明确外泌体的具体调控机制,为后期 ACS 的诊断提供更有力的参考依据。

#### 参考文献:

- [1] 刘永玲,罗厚龙,刘行超,等.血清 SOD 和 hsCRP 等指标在急性冠脉综合征中的应用及相关性分析[J].现代检验医学杂志,2017,32(6): 115-117, 121. LIU Yongling, LUO Houlong, LIU Xingchao, et al. Level and correlation of serum SOD and hsCRP and other indicators in acute coronary syndrome [J]. Journal of Modern Laboratory Medicine, 2017, 32(6): 115-117, 121.
- [2] LEE J, KIISKINEN T, MARS N, et al. Clinical conditions and their impact on utility of genetic scores for prediction of acute coronary syndrome[J]. Circulation. Genomic and Precision Medicine, 2021, 14(4): e003283.
- [3] ZAMANI P, FEREDOUNI N, BUTLER A E, et al. The therapeutic and diagnostic role of exosomes in cardiovascular diseases[J]. Trends in Cardiovascular Medicine, 2019, 29(6): 313-323.
- [4] ZHANG Yunyuan, LUN Limin, LI Hui, et al. The value of lncRNA NEAT1 as a prognostic factor for survival of cancer outcome: a meta-analysis[J]. Scientific Reports, 2017, 7(1): 13080.
- [5] KOYAMA R, MANNIC T, ITO J, et al. MicroRNA-204 is necessary for aldosterone-stimulated T-type calcium channel expression in cardiomyocytes[J]. International Journal of Molecular Sciences, 2018, 19(10): 2941.
- [6] SANTANA I V, TANUS-SANTOS J E. Serum or plasma matrix metalloproteinase (MMP)-9 levels and cardiovascular diseases[J]. Journal of Cardiovascular Translational Research, 2018, 11(6): 524-525.
- [7] CREA F, LIBBY P. Acute coronary syndromes: the way forward from mechanisms to precision treatment[J]. Circulation, 2017, 136(12): 1155-1166.
- [8] HENNING R J. Cardiovascular exosomes and microRNAs in cardiovascular physiology and pathophysiology[J]. Journal of Cardiovascular Translational Research, 2021, 14(2): 195-212.
- [9] MA Min, HUI Jie, ZHANG Qiyin, et al. Long non-coding RNA nuclear-enriched abundant transcript 1 inhibition blunts myocardial ischemia reperfusion injury via autophagic flux arrest and apoptosis in streptozotocin-induced diabetic rats[J]. Atherosclerosis, 2018, 277(9): 113-122.
- [10] WANG Lei, XIA Jingwen, KE Zunping, et al. Blockade of NEAT1 represses inflammation response and lipid uptake via modulating miR-342-3p in human macrophages THP-1 cells [J]. Journal of Cellular Physiology, 2019, 234(4): 5319-5326.
- [11] LUO Man, SUN Qingsong, ZHAO Hongmei, et al. Long noncoding RNA NEAT1 sponges miR-495-3p to enhance myocardial ischemia-reperfusion injury via MAPK6 activation[J]. Journal of Cellular Physiology, 2020, 235(1): 105-113.
- [12] LU Yaoyong, LI Tao, WEI Ganbao, et al. The long non-coding RNA NEAT1 regulates epithelial to mesenchymal transition and radioresistance in through miR-204/ZEB1 axis in nasopharyngeal carcinoma[J]. Tumor Biology, 2016, 37(9): 11733-11741.
- [13] LIU Jing, LIU Yong, WANG Feng, et al. MiR-204: molecular regulation and role in cardiovascular and renal diseases[J]. Hypertension, 2021, 78(2): 270-281.
- [14] DU Youyou, LIU Ganghui, ZHAO Luosha, et al. Protective effect of miR-204 on doxorubicin-induced cardiomyocyte injury via HMGB1[J]. Oxidative Medicine and Cellular Longevity, 2020, 2020: 8819771.
- [15] 吴静,燕成英,温元善,等.血清 miR-9,miR-204 水平对 PCI 术后 ACS 患者预后的评估价值[J].心血管康复医学杂志,2021,30(2): 143-147. WU Jing, YAN Chengying, WEN Yuanshan, et al. The value of the expressions of miR-9 and miR-204 in the prognosis of acute coronary syndrome [J]. Chinese Journal of Cardiovascular Rehabilitation Medicine, 2021, 30(2): 143-147.
- [16] LUAN Wenkang, QIAN Yao, NI Xin, et al. MiR-204-5p acts as a tumor suppressor by targeting matrix metalloproteinases-9 and B-cell lymphoma-2 in malignant melanoma[J]. Onco Targets & Therapy, 2017, 10(2): 1237-1246.
- [17] WANG Xi, KHALIL R A. Matrix metalloproteinases, vascular remodeling, and vascular disease[J]. Advances in Pharmacology (San Diego, Calif.), 2018, 81: 241-330.



- [18] NANDI S S, KATSURADA K, SHARMA N M, et al. MMP-9 inhibition increases autophagic flux in chronic heart failure[J]. American Journal of Physiology-Heart and Circulatory Physiology, 2020, 319(6): H1414-H1437.
- [19] 陈俊, 廖应英, 孙泽群. 基质金属蛋白酶-9与心房颤动的关系及对持续性心房颤动患者复律治疗后复发的预测价值[J]. 中国循环杂志, 2017, 32(1): 67-71. CHEN Jun, LIAO Yingying, SUN Zequn, et al. Relationship between matrix metalloproteinase-9 level and atrial fibrillation with its predictive value of AF recurrence in persistent AF patients after cardio-version[J]. Chinese Circulation Journal, 2017, 32(1): 67-71.
- [20] YU Yang, WANG Leilei, LIU Tong, et al. MicroRNA-204 suppresses trophoblast-like cell invasion by targeting matrix metalloproteinase-9[J]. Biochemical and Biophysical Research Communications, 2015, 463(3): 285-291.
- 收稿日期: 2022-03-28  
修回日期: 2022-09-14

(上接第48页)

- [30] MAES M, SUPASITTHUMRONG T, LIMOTAI C, et al. Increased oxidative stress toxicity and lowered antioxidant defenses in temporal lobe epilepsy and mesial temporal sclerosis: Associations with psychiatric comorbidities[J]. Mol Neurobiol, 2020, 57 (8):3334-3348.
- [31] LU Wei, WU Zhangze, ZHANG Chong, et al. Jujuboside A exhibits an antiepileptogenic effect in the rat model via protection against traumatic epilepsy-induced oxidative stress and inflammatory responses[J]. Evid Based Complement Alternat Med, 2022, 2022:7792791.
- [32] RAMAZI S, FAHANIK-BABAEI J, MOHAMADI-ZARCH S M, et al. Neuroprotective and anticonvulsant effects of sinomenine in kainate rat model of temporal lobe epilepsy: Involvement of oxidative stress, inflammation and pyroptosis[J]. J Chem Neuroanat, 2020, 108:101800.
- [33] PAULETTI A, TERRONE G, SHEKH-AHMAD T, et al. Targeting oxidative stress improves disease outcomes in a rat model of acquired epilepsy[J]. Brain, 2019, 142 (7):e39.
- [34] LIANG Liping, WALDBAUM S, ROWLEY S, et al. Mitochondrial oxidative stress and epilepsy in SOD2 deficient mice: attenuation by a lipophilic metalloporphyrin[J]. Neurobiol Dis, 2012, 45 (3):1068-1076.
- [35] WEN Fang, TAN Zhigang, XIANG Jun. Cu-Zn SOD suppresses epilepsy in pilocarpine-treated rats and alters SCN2A/Nrf2/HO-1 expression[J]. Epileptic Disord, 2022, 24 (4):647-656.
- [36] LIANG Liping, PATEL M. Mitochondrial oxidative stress and increased seizure susceptibility in Sod2(-/+) mice[J]. Free Radic Biol Med, 2004, 36 (5):542-554.
- [37] LORIGADOS PEDRE L, GALLARDO J M, MORALES CHACON L M, et al. Oxidative stress in patients with drug resistant partial complex seizure[J]. Behav Sci (Basel), 2018, 8 (6):59.
- [38] KALITA J, MISRA U K, SINGH L S, et al. Oxidative stress in status epilepticus: A clinical-radiological correlation[J]. Brain Res, 2019, 1704: 85-93.
- [39] ELKOMMOS S, MULA M. Current and future pharmacotherapy options for drug-resistant epilepsy[J]. Expert Opin Pharmacother, 2022, 23(18):2023-2034.
- [40] MCDONALD T S, NEAL E S, BORGES K. Fructose 1,6-bisphosphate is anticonvulsant and improves oxidative glucose metabolism within the hippocampus and liver in the chronic pilocarpine mouse epilepsy model[J]. Epilepsy Behav, 2021, 122:108223.
- [41] COBLEY J N, FIORELLO M L, BAILEY D M. 13 reasons why the brain is susceptible to oxidative stress[J]. Redox Biol, 2018, 15:490-503.
- [42] POPOVA I, MALKOV A, IVANOV A I, et al. Metabolic correction by pyruvate halts acquired epilepsy in multiple rodent models[J]. Neurobiol Dis, 2017, 106:244-254.
- [43] MINENKOVA A, JANSEN E E W, CAMERON J, et al. Is impaired energy production a novel insight into the pathogenesis of pyridoxine-dependent epilepsy due to biallelic variants in ALDH7A1? [J]. PLoS One, 2021, 16(9):e0257073.
- [44] FEI Yaqing, SHI Ruting, SONG Zhi, et al. Metabolic control of epilepsy: A promising therapeutic target for epilepsy[J]. Front Neurol, 2020, 11:592514.
- [45] 王丽丽, 张宁, 赵迎春, 等. 老年急性脑出血并发癫痫患者血清HP, SOD, MDA水平表达及其与认知功能损害的相关性研究[J]. 现代检验医学杂志, 2020, 35(2):108-111. WANG Lili, ZHANG Ning, ZHAO Yingchun, et al. Study on the correlation between serum haptoglobin, superoxide dismutase and malondialdehyde in elderly patients with epilepsy after acute cerebral hemorrhage and their correlation with cognitive impairment[J]. J Mod Lab Med, 2020, 35(2):108-111.
- [46] QIAN Xudong, XU Qianqian, LI Guoyun, et al. Therapeutic effect of idebenone on rats with vascular dementia via the microRNA-216a/RSK2/NF- $\kappa$ B axis[J]. Neuropsychiatr Dis Treat. 2021, 17:533-543.
- [47] LIN Pengfei, LIU Junling, REN Ming, et al. Idebenone protects against oxidized low density lipoprotein induced mitochondrial dysfunction in vascular endothelial cells via GSK3 $\beta$ / $\beta$ -catenin signalling pathways[J]. Biochem Biophys Res Commun, 2015, 465(3):548-555.
- 收稿日期: 2022-10-11  
修回日期: 2023-01-03