医学拾萃

IMPROVE REVIEW

标本处理系统篇
Pre-Analytical System

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IMPROVE 陽普醫療 IMPROVE MEDICAL

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文献导读

本期的文献导读是一篇关于《凝血途径及不同促凝剂对检测的影响》综述，介绍了凝血机制的内源性凝血途径和外源性途径，不同促凝剂对检测的影响，促凝管中纤维蛋白析出的原因及解决办法，以期帮助临床理解和分析含有促凝剂的采血管对检测的影响，并制定针对性的解决措施。

凝血途径及不同促凝剂对检测的影响

1. 凝血途径

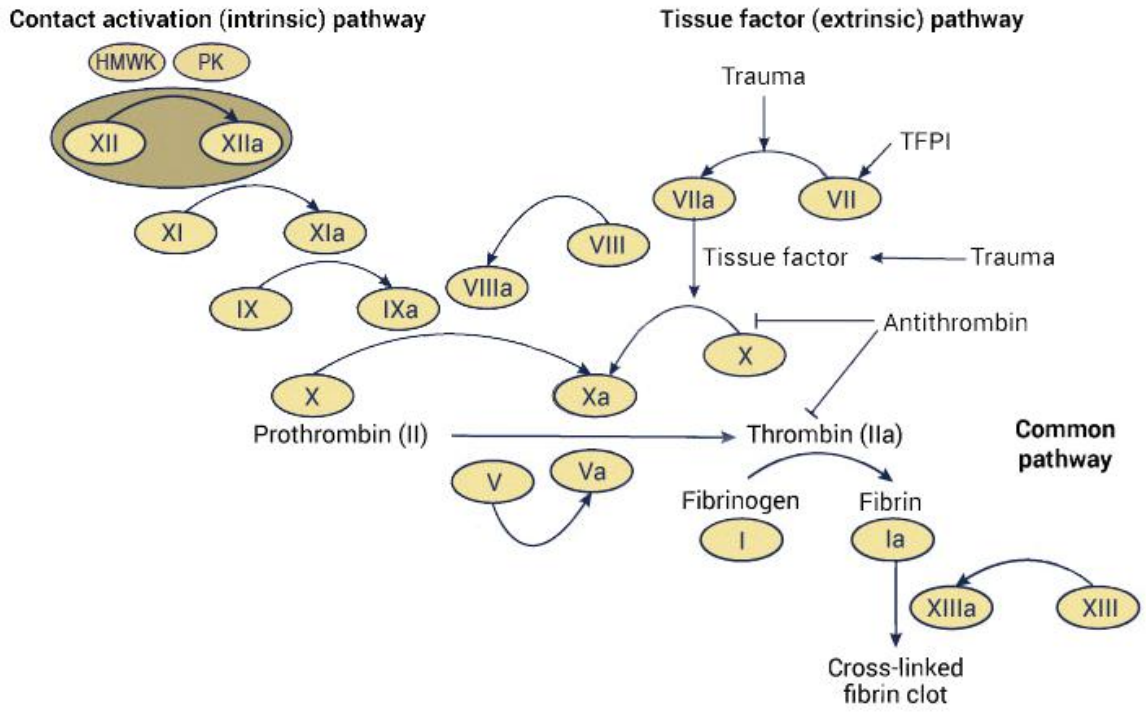
血液的凝固是由许多凝血因子参与的酶促反应。由血浆中可溶性的纤维蛋白原转变成不可溶的纤维蛋白，导致凝血。凝血途径分为内源性和外源性凝血途径[1-3]。

内源性途径是指由血管内的创伤激活的蛋白质相互作用的多个级联，它也被血小板、暴露的内皮或胶原激活，通常需要时间才能形成血凝块。参与血凝块形成的蛋白质称为凝血因子，内源性途径中涉及凝血因子 VIII、IX、XI 和 XII，通过因子 XII 与暴露于血液的带负电荷的异物表面结合而被激活，依次激活因子 IX、X 和 XI，进一步激活将凝血酶原转化为凝血酶的因子 II。凝血酶将纤维蛋白原转化为纤维蛋白。血小板被困在纤维蛋白网内，形成血块。

外源性途径是指被损坏的外表面激活的蛋白质相互作用的多个级联。因子 III 和凝血活酶参与外源性途径。外源性途径比内源性途径短，并且比内源性途径快。凝血活酶是在正常情况下不会暴露于血液的组织因子（TF）。但是在血管或内皮细胞损伤下，凝血活酶的暴露会激活因子 VIIa 和磷脂[1-2]，从而转化为因子 IX。最后，因子 X 被来自外源性途径的因子 Xa 激活[3]。

两种凝血途径的区别

区别	内源性途径	外源性途径
定义	由血管内的创伤激活的蛋白质相互作用的多个级联	由受损的外表面激活的蛋白质相互作用的多个级联
激活	由内伤激活	由外伤激活
凝血因子	因子 VIII、IX、XI 和 XII	因子 VII
凝血效率	慢	快
启动凝血途径需要的时间	大约需要 15～20 秒开始凝血	大约需要 2～6 分钟才能开始凝血



凝血瀑布

2. 促凝剂类型

内源性的凝血途径被激活需要与体外物质接触而启动[4,5]，通常是带负电荷的异物表面。玻璃、硅石、白陶土、皂粘土或者是硅藻土都可以作为快速促凝剂[6]。而微小的促凝剂像无机硅酸盐类相对慢些（30 ~ 60 分钟）[7,8]。40% ~ 80%的管壁都会覆盖二氧化硅促凝剂[9-11]。二氧化硅促凝剂通常是球体的，直径范围是 0.01 ~ 100 μm（比较适合的是 0.4 ~ 20 μm）[5]。在分离的血清中，促凝剂还有一个优点就是减少潜在的纤维蛋白[12]。

第二种激活促凝因子的途径是外源性途径，是一种生物化学反应，并且和浓度有关，生化促凝剂像鞣花酸、凝血酶、蛇毒液及促凝血酶原激酶等[7,13-15]被加工成小颗粒或者是纸盘或者是喷到试管的表面，试管的表面含有像聚维酮、羧甲基纤维素、聚乙烯醇或者是聚氧化乙烯一样的水溶性物质作为载体。

3. 促凝剂对检测的影响

3.1 对激素检测的影响

含有促凝因子的采血管里检测的睾酮浓度高于正常检测的四倍[16]。对所选的标本进行监测，促凝剂干扰睾酮检测的方法中离子对 m/z 的比率是 289.3/97.1[16]。改变离子对 m/z 的比率为 289.2/109.0 就可以消除这种干扰[16]。

3.2 对质谱法检测的影响

在含有促凝剂的采血管里通过质谱法获得蛋白质的含量会有所改变[17,18]。二氧化硅和硅酸盐促凝剂可以诱导体内和体外的蛋白质释放、激活、形成基质金属蛋白酶-9 复合物（明胶酶-B）[19,20]。存在二氧化硅和锌离子情况下，红细胞释放基质金属蛋白酶-9 时可导致基质金属蛋白酶-9 浓度升高、血块激活[18,19]。为避免这个问题，有研究推荐采用柠檬酸盐抗凝血浆检测基质金属蛋白复合物-9[21]。

有研究显示，血浆与血清中代谢物浓度有明显差异[22-25]，基于核磁共振的代谢组学研究证明等离子体、EDTA、肝素和柠檬酸盐抗凝血剂会严重影响代谢信息的复原[26,27]。采血管中的添加剂对多肽峰的检测具有明显的影响，原因是抗凝或促凝剂激活的凝血过程不同，蛋白在此过程中的水解片段也不同；凝血与纤溶的平衡是动态的，这一过程是一系列的酶促反应，蛋白酶的连续激活与失活，血小板失活导致凝块形成，这些都有可能释放出肽和蛋白质片段[28,29]。在血清中，代谢物的浓度要普遍高于血浆，但两者仍呈现明显的相关性[30,31]。Breier 等[32]的研究指出，血清中 101 种代谢物浓度显著高于血浆，血清中代谢物的可靠性更高，推荐血清管用于质谱法检测。

3.3 对凝血试验的影响

2003 年以前，美国 CLSI 建议排管顺序凝血管放在血清管

后面采血[33]，但在 H3-A5 版本及后续版本中修改为凝血管放在血清管前面[34]，主要是为了避免血清管中促凝剂污染凝血管，促凝剂可能会影响凝血试验结果。Yoko Fukugawa 等[35]研究了血清管中促凝剂污染对主要凝血试验结果的影响，发现凝血酶血清管放在凝血管前采血会影响大部分凝血试验，尤其是 APTT 和 FMC 显著发生变化；二氧化硅血清管影响部分凝血试验（主要是 PT 值），变化不显著；建议采用标准化采血程序，最大程度减少血清促凝剂对凝血试验的影响[35]。

添加不同量的促凝剂改变凝血测试结果（平均值 ± 标准偏差）[35]

促凝剂溶液								
采血管	体积(μL)*	PT (%)	PT (Ratio)	PT-INR	APTT(s)	纤维蛋白原 (mg/dL)	DD(μg/mL)	FMC (μg/mL)
T1	2	104.8 ± 8.8†	0.980 ± 0.04†	0.975 ± 0.05†	34.0 ± 2.3§	271.4 ± 46.9	0.21 ± 0.11†	48.30 ± 34.13§
	5	105.2 ± 8.1‡	0.978 ± 0.04†	0.971 ± 0.04‡	32.5 ± 2.3§	269.3 ± 49.2	0.31 ± 0.24	124.19 ± 55.76§
	10	104.7 ± 8.8‡	0.981 ± 0.04†	0.975 ± 0.05†	31.2 ± 2.8§	248.9 ± 58.3‡	0.41 ± 0.48†	176.22 ± 27.04§
S1	2	105.3 ± 9.2§	0.979 ± 0.04§	0.972 ± 0.05§	35.8 ± 2.5	270.3 ± 51.0	0.20 ± 0.11	3.45 ± 0.96
	5	105.1 ± 8.4§	0.979 ± 0.04§	0.973 ± 0.04§	35.5 ± 2.6	272.6 ± 50.0	0.20 ± 0.12	4.24 ± 1.41
	10	105.0 ± 8.3§	0.979 ± 0.04§	0.973 ± 0.04§	35.7 ± 2.5	271.5 ± 45.6	0.19 ± 0.10	3.60 ± 1.27
S2	2	106.0 ± 9.1§	0.976 ± 0.04§	0.968 ± 0.05§	35.7 ± 2.8	273.5 ± 46.1	0.20 ± 0.10†	3.87 ± 1.61
	5	105.8 ± 8.8§	0.975 ± 0.04§	0.969 ± 0.04§	35.4 ± 2.6†	273.1 ± 48.7	0.19 ± 0.09	3.69 ± 1.11
	10	105.7 ± 8.5§	0.976 ± 0.04§	0.969 ± 0.04§	35.4 ± 2.5‡	272.1 ± 46.5	0.21 ± 0.11	3.72 ± 1.34
对照	2	102.8 ± 8.0	0.988 ± 0.04	0.984 ± 0.04	35.8 ± 2.6	273.5 ± 48.4	0.24 ± 0.12	3.27 ± 1.48
(盐水)	5	102.8 ± 7.7	0.990 ± 0.04	0.985 ± 0.04	35.7 ± 2.6	270.6 ± 47.9	0.24 ± 0.14	3.73 ± 1.99
	10	102.7 ± 7.80	0.989 ± 0.04	0.985 ± 0.04	35.8 ± 2.5	268.2 ± 44.9	0.20 ± 0.10	3.54 ± 1.63

APTT，活化部分凝血活酶时间；DD，D-二聚体；FMC，纤维蛋白单体复合物；PT，凝血酶原时间；PT-INR，凝血酶原时间国际标准化比率；S1，仅玻璃颗粒；S2，仅二氧化硅颗粒；T1，凝血酶+玻璃颗粒。

*添加至每支血凝管中钟的促凝剂体积

† $p < 0.05$

‡ $p < 0.01$

§ $p < 0.001$ (与对照相比)

3.4 对其他检测的影响

Sampson 等[36]通过 Lytening 2Z 离子选择电极分析仪，发现二氧化硅或是聚硅酮表面活化剂可导致锂离子浓度假性升高。RST 凝血酶管凝血时间 2.49 分钟，SST 分离胶管凝血时间 19.47 分钟，两种管的血糖、钙、LD 检测值具有统计学显著差异，葡萄糖和 LD 的差异也具有临床差异，RST 凝血酶管不适用于 LD 和血糖检测[37]。对于接受高剂量肝素或华法林治疗的患者，不适合使用 RST 凝血酶管，因为管中潜在的凝块形成可能会堵塞仪器探针并产生错误的测试结果[38]。基于这些发现，显然需要进一步的研究以确保凝血管给予其他市售血清管临床上相同的结果，特别是采血量不足的采血管[38]。

4. 促凝管纤维蛋白二次析出的原因及解决办法

在化学发光免疫检测和 ELISA 中，标本的凝血时间、凝血温度、离心时间、离心力（转速）等对 TnI、HBsAg、Anti-HIV、HBeAg 等项目均造成假阳性结果，引起跳值。Goce 的研究表明 BD 和非可替的血清管和肝素管在 Access 2 和 DXI800 上检测的 TnI 假阳性率约为 14/1000[39-42]，引起跳值的可能物质是纤维蛋白、脂类复合物、细胞碎片等[39-43]。

肝素锂血浆中不溶性纤维蛋白对 Beckman AccuTnI 测定的影响[39]

纤维蛋白样本	cTnI，mg/L	实际检测时间
纤维蛋白等分试样 1	0.061	17：45
纤维蛋白等分试样 1(reflex)	0.016	17：59
纤维蛋白等分试样 2	0.050	17：46
纤维蛋白等分试样 2(reflex)	0.023	18：00
纤维蛋白等分试样 3	0.038	17：47
纤维蛋白等分试样 4	0.035	17：47
纤维蛋白等分试样 5	0.034	17：48
离心等分试样	0.015	18：00
离心等分试样	0.013	18：14

促凝管中纤维蛋白二次析出的原因通常有：凝血不充分、温度过低导致凝血时间延长（凝血不充分）、离心力过小或转

速不够、以及凝血酶缺乏者或肝素抗凝治疗病人凝血功能障碍等。可以通过确保采血量足够、采血后及时轻轻颠倒摇匀、延长标本凝血时间、彻底去除肉眼可见纤维蛋白、增加离心力和转速[44]、延长标本离心时间、重复离心[44,45]等方式解决血清管中纤维蛋白二次析出问题。

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Article Abstract Collection

文献摘要

本期的文献摘要，选取了综述中若干重要的参考文献，针对其摘要做了中文翻译。这些文献综述或研究了血栓形成的原因、真空采血管的设计、真空采血管内壁涂层对血液标本的影响、不同添加剂对凝血检测的影响、促凝剂对激素检测和质谱法检测以及对其他检测项目的影响。

参考文献摘要翻译

1. **Owens AP, Mackman N. Tissue factor and thrombosis: The clot starts here. Thromb Haemost 2010;104:432-439.**

摘要

血栓形成或其并发症目前在发达国家的心血管病发病率和死亡率中排名前三位。预防和治疗血栓形成的安全有效药物数量有限。为了更好地了解血凝块形成过程中涉及的复杂成分和相互作用，血栓形成的动物模型必不可少。组织因子（TF）是开始凝血的必需元素，可能在动脉和静脉血栓形成中起关键作用。了解 TF 在血栓形成中的作用可能会允许开发新的抗血栓药物。本综述将聚焦 TF 在体内血栓形成模型中的作用。

2. **Augello FA, Rainen L, Walenciak M, Oelmuller U, wyrich R, Bastian H. “Method and device for collecting and stabilizing a biological sample”, U.S. Patent No.6,602,718, November 2000.**

摘要

一种用于采集预定体积的生物样品（特别是全血样品）的采集容器和方法，包含有效量的至少一种稳定剂。稳定剂能够在采集时稳定生物样品中的核酸，以防止核酸的酶促降解。稳定剂包括阳离子化合物、洗涤剂（特别是阳离子洗涤剂）、离液盐、核糖核酸酶抑制剂、螯合剂，以及这些物质的混合物。

3. **Dubrowny NE, Harrop AJ. “Collection device”, U.S. Patent No.6,686,204, February 2004.**

摘要

本发明是一种容器组件，其包括在容器组件的内表面上的涂层成分，更具体地，包括用于在采血管内快速凝结血样的涂层成分。该涂层成分包括内源性凝血活化剂和外源性凝血活化剂，用于活化内源途径和外源途径以促进血液样品的快速凝结。此外，本发明还包括用于封闭容器组件的阻挡包装。

4. **Ali K. “Methods for improving uniformity of silica films on substrates”, U.S. Patent No.4,420,517, May 1982.**

摘要

提供了一种用于增强基材（例如，真空采血管）上的二氧化硅涂层的均匀性的方法，该均匀性的提高导致二氧化硅的有效表面积显著增加，从而改善了凝结活化。该方法包括在酸或碱溶液的存在下水解硅酸乙酯和异丙醇的混合物以获得二氧化硅沉淀。将所得的硅溶胶涂覆到基材上，并在低加热程序的存在下进行干燥。或者，如果二氧化硅需要释放到水基物质中，例如随后需要在涂覆的容器中引入血液，如果有需要，可以将二氧化硅溶胶与水溶性载体（如聚乙烯基吡咯烷酮）混合，然后再施用于基材上。

5. **Vogler EA, Harper GR, Graper JC. “Vacuum actuated blood collection assembly including tube of clot-accelerating plastic”, U.S. Patent No.5,344,611, September 1994.**

摘要

一种真空的血液采集组件，包括具有经等离子体处理的内壁表面和被可刺穿隔膜覆盖的开口端的塑料容器。内壁表面也可以是磨砂的，用于增加表面积。本发明包括一种制造该组件的方法。

6. **Kasai M, Yamazaki S. “Blood collection tube”, U.S. Patent No.5,213,765, May 1993.**

摘要

采血管包括底部和被塞子密封的开口端。管内涂覆含抗凝剂的支撑膜。支撑构件的抗凝剂涂覆表面设置有大量的细微凸起。

7. **Vogler E, Graper J. “Dual pathway clotting enhancer for blood collection tube”, U.S. Patent No.5,378,431, June 1993.**

摘要

一种血液采集组件，包括一种可选择地由可刺穿的隔膜覆盖并抽真空的容器。容器中的凝血增强剂可激活内源性和外源性凝血途径。

8. **Kessler SB. “Method for collection blood”, U.S. Patent No.4,153,739, June 1977.**

摘要

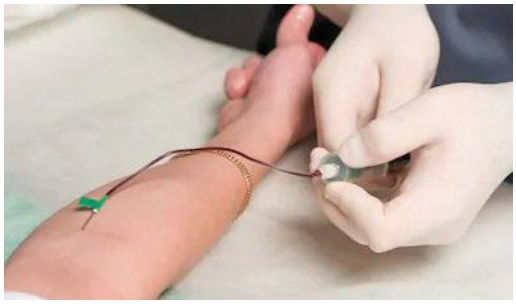
本公开专利涉及一种维持血液采集组件中的硅化表面的血液促凝特性的方法。该方法包括用水溶性惰性成膜屏障材料涂覆硅化表面。本发明方法的优点是保留了快速的凝结时间。本发明还涉及改进的血液采集组件，在该组件中，凝块激活部件涂覆含水溶性惰性聚合物材料的膜屏障。

9. **Wang C, Shiraishi S, Leung A, et al. Validation of a testosterone and dihydrotestosterone liquid chromatography tandem mass spectrometry assay: interference and comparison with established methods. Steroids 2008;73:1345-1352 Epub 2008 May 21.**

摘要

睾酮（T）及其代谢物二氢睾酮（DHT）是具有不同生物学特性的雄激素。在进行 5α 还原酶治疗前列腺疾病和使用新的雄激素制剂期间，需要进行 T 和 DHT 测量，以评估患有性别畸形和多毛症的患者。我们的实验室开发并验证了一种通过液相色谱串联质谱法（LC-MS/MS）同时测定临床化学实验室血清 T 和 DHT 的方法。从使用含有促凝剂的试管采集的血液中的血清分析得出的 T 值比普通试管采集的血液中的高四倍。改变选择用于监测的离子对可消除促凝剂的干扰。在涂有氟化物的试管中采集的血液的血清 T 和 DHT 水平分别比在

普通试管（无添加剂）中测量的水平低 20%和 15%。由于红细胞中非特异性酯酶的作用，在普通管采集的血液中添加 T 庚酸酯会引起剂量相关的血清 T 水平升高。通过使用氟化物管进行血液采集可以避免这种酯酶活性。用 LC-MS/MS 测定的血清 DHT 水平始终低于放射免疫测定法。当血清 DHT 浓度低时，差异是浓度依赖性的，且差异的方差较大。放射免疫测定之前的硅藻土色谱仪减少了两种方法之间的差异，因此证实了通过免疫测定获得的较高的 DHT 含量可能是由于被硅藻土色谱法部分去除的干扰物质造成的。



10. **Banks RE, Stanley AJ, Cairns DA, Barrett JH, Clarke P, Thompson D, et al. Influences of blood sample processing on low-molecular weight proteome identified by surface-enhanced laser desorption/ionization mass spectrometry. Clin Chem 2005;51:637-649.**

摘要

蛋白质组学中的剖析方法 [例如表面增强的激光解吸/电离（SELDI）质谱] 可用于疾病标记物的发现。本研究的目的是调查所选分析前因素对所得结果的潜在影响。将 EDTA、柠檬酸盐或肝素抗凝的血浆样品以及健康志愿者的血清样品通过 SELDI 在 CM10 [固定有铜和 H50 芯片表面的金属亲和力捕获（IMAC）阵列] 上进行分析。使用线性混合效应模型，我们检查了静脉穿刺和样品分离之间经历的时间（0 ~ 24 小时）以及所用血清管类型（Greiner Vacuette 促凝管、分离胶促凝管或无添加剂管）的影响。我们分析了纯化的血小板以及用钙和/或凝血酶处理的贫血小板和富血小板血浆样品，以确定直接或通过凝血过程产生的血小板对生成图谱的贡献。然后，我们使用聚类分析来鉴定具有相似特征峰的样品。不同的血浆类型和血清可以基于它们的光谱图谱的聚类分析来区分。从静脉穿刺到从血液样品中分离血浆和血清之间经过的时间改变了所获得的曲线，特别是对于血清样品，尤其是在 IMAC 芯片上。由于凝血时间的差异，血清采集管的类型也会影响图谱。血小板的体外处理显示，血清 IMAC 谱中的特定峰似乎直接来自血小板。在凝血过程中还出现了其他一些峰，包括一些随时间变化的峰。分析前变量（例如样品处理）会明显影响结果。

11. **Mannello F, Tanus-Santos JE, Meschiari CA, Tonti GA. Differences in both matrix metalloproteinase 9 concentration and zymographic profile between plasma and serum with clot activators are due to the presence of amorphous silica or silicate salts in blood collection devices. Anal Biochem 2008;374:56-63.**

摘要

基质金属蛋白酶（MMP）是很有前景的诊断工具，血液采样/处理可改变血浆与血清之间以及含或不含促凝剂的血清之间的 MMP 浓度。为了解释促凝管采集的血清相对于血浆和无添加剂管采集的血清中较高的 MMP-9 表达，我们分析了来自 50 位健康捐献者，用塑料管和玻璃管采集的柠檬酸盐血浆、血清和白细胞层中二氧化硅和硅酸盐（凝块活化剂的成分）增加的影响，我们还分析了二氧化硅和硅酸盐对培养的白血病细胞的影响。在玻璃管和塑料管之间，血清和血浆之间，有和没有促凝剂的血清之间，或在二氧化硅和硅酸盐处理之间，MMP-2 的水平均未显示出明显变化。通过向先前分离的血浆和血清中添加二氧化硅或硅酸盐，未获得 MMP-9 表达的修饰。相对于血清和柠檬酸盐血浆，在血液采集之前增加添加至柠檬酸盐管和无添加剂管中的不溶性二氧化硅和可溶性硅酸盐的量会导致 MMP-9 含量增加。添加到白细胞层和白血病细胞中的二氧化硅和硅酸盐显著诱导了 MMP-9 的释放/分泌，表明二氧化硅和硅酸盐都诱导了前 MMP-9 和复合 MMP-9 形式的释放。我们建议限制血清的滥用，并避免促凝剂的干扰作用。

12. **Liu X, Hoene M, Wang X, et al. Serum or plasma, what is the difference? Investigations to facilitate the sample material selection decision making process for metabolomics studies and beyond[J]. Analytica Chimica Acta, 2018, 1037 : 293-300.**

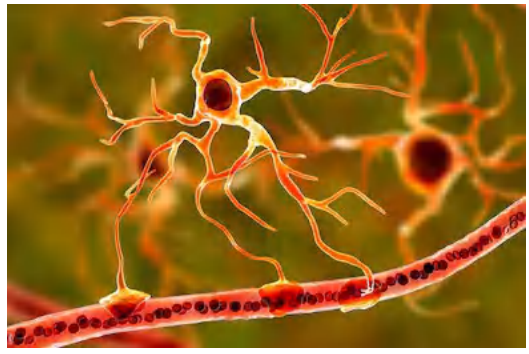
摘要

在分析化学中，推荐血清和血浆作为样品材料。然而，血液处理产生的血清或血浆是存在差异的。血浆或血清是否是较好的样本材料仍有争议。我们在配对样本中进行了三项 UHPLC-质谱驱动的代谢组学研究。在研究 1 中，比较了血清和血浆的代谢物谱。216 种代谢产物中有 46%表现出显著差异的水平（配对 Wilcoxon 符号秩检验，$p < 0.05$，FDR < 0.01），只有 3 种代谢产物（蛋氨酸、C2:0-和 C3:0-肉碱）表现出较低的水平。在研究 2 中，对三种不同的血清采集管的比较显示，凝血和相关过程明显地改变了代谢产物的水平，这取决于不同采血管特定的凝血过程。二肽苯丙氨酸苯丙氨酸（在含硅酸盐的血清采集管中含量最高）的差异最为显著。在研究 3 中，研究了血小板可能的不良反应，即使经过正确的处理，血小板仍然留在标准血浆中。在标准血浆和无血小板血浆（PFP）的比较中，216 种代谢物的模式没有差异。我们的结果对血清和血浆之间的基本差异提供了新的见解，从而为分析化学家在开始复杂和耗时的分析研究之前决定使用血清或血浆提供了有价值的信息。

13. **Nishiumi S, Suzuki M, Kobayashi T, et al. Differences in metabolite profiles caused by pre-analytical blood processing procedures[J]. J Biosci Bioeng, 2018, 125(5): 613-618.**

摘要

近年来，在临床研究中，利用人血清和血浆的代谢组学分析进行生物标志物发现和疾病诊断的方法越来越多。使用代谢物生物标记物进行疾病诊断的可行性在很大程度上取决于分析前血液处理过程中代谢物的稳定性，如血清或血浆采样和离心前样品储存。然而，血液处理过程对代谢物稳定性的影响尚未完全阐明。本研究采用气相色谱-质谱联用技术和液相色谱-质谱联用技术比较了人血清和血浆中代谢物的含量。此外，我们还评估了离心前在室温或低温下储存引起的血浆代谢物水平的变化。结果发现，76 种代谢物在血清和血浆水平上存在显著差异。此外，离心前储存条件显著影响血浆中 45 种代谢物的水平。这些结果强调了代谢组学分析中血液处理程序的重要性，在发现生物标记物和随后使用生物标记物进行疾病诊断时应考虑到这一点。



14. **Zhou Z, Chen Y, He J, et al. Systematic evaluation of serum and plasma collection on the endogenous metabolome[J]. Bioanalysis, 2017, 9(3): 239-250.**

摘要

目的：在代谢组学研究中，采用不同的采血方法可能会影响内源性代谢产物。

材料与方法：采用超高效液相色谱、质谱联用和多元统计分析研究不同抗凝剂处理的血清和血浆标本的代谢组学差异。共评估了 135 种已知的代表性代谢产物，进而对抗凝剂效果进行综合评价。

结果：血清采集管分离凝胶成分和抗凝剂等外因影响质谱检测。肝素血浆对不同功能组的检测效果最好，因此是代谢组学研究的最佳血样，其次是草酸钾血浆。

15. **Ilies M, Iuga C A, Loghin F, et al. Impact of blood sample collection methods on blood protein profiling studies[J]. Clinica Chimica Acta, 2017, 471: 128-134.**

摘要

分析前因素对用于定性和定量蛋白质分析的血液样本的完整性有显著影响。重要的因素是采血管的类型和使用的抗凝剂。只有少数研究通过比较血清和 EDTA 血浆采集管与一些靶蛋白或肽来评估这些变量。在这项研究中，我们调查了在血清、EDTA、肝素和柠檬酸盐血浆管中采集的血液样本的蛋白质谱。此外，我们还通过可检测蛋白质组的血液蛋白质谱、方差和覆盖率，比较了 6 种高丰度蛋白质的损耗效率。血清和血浆中的肽数和经典血液蛋白的出现率差异最大。肝素血浆显示了大量可检测蛋白，整体方差较低，与 EDTA-和柠檬酸盐血浆高度相似，因此也可能是一种有用的血液蛋白分析试管。此外，还描述了一组核心的血液蛋白质，并揭示了样本特定蛋白质的部分和组成。因此，蛋白质分析研究应考虑样品采集方法等分析前问题。

16. **Hebels DG, Georgiadis P, Keun HC, et al. Performance in omics analyses of blood samples in long-term storage: opportunities for the exploitation of existing biobanks in environmental health research[J]. Environ Health Perspect, 2013, 121(4): 480-487.**

摘要

对过去几十年收集的、目前储存在生物库中的生物样本进行组学分析的适用性尚不清楚。我们评估了血液衍生生物样本的处理和储存条件对转录组学、表观基因组学（CpG 甲基化）的影响，血浆代谢组学 [UPLC-ToFMS（超高效液相色谱飞行时间质谱法）] 和宽靶蛋白组学。我们收集了新鲜血液样品，置于不含 RNA 防腐剂的肝素、EDTA 或柠檬酸盐中，并在室温下保存了不超过 24 小时，然后将其分离成白膜层、红细胞和血浆在 -80 摄氏度或液氮中冷冻馏分。我们开发了从白膜层中分离 RNA 的方法，并进行了组学分析。最后，我们分析了来自意大利 EPIC 和瑞典北部的健康与疾病研究生物库的类似样本。采集的血液在冷冻 8 小时内通过在 RNA 防腐剂中解冻获得样本，微阵列质量的 RNA 可以从（解冻后样本的）白膜层（包括大多数生物库的样本）中分离出来。不同抗凝剂对代谢组学、蛋白质组学和转录组学的影响较小。血液分馏前的延迟（仅 2 小时）对转录组的影响最大，而储存温度的影响最小。收集后 8 小时以上处理的样品对代谢组学和蛋白质组学有影响，但不受储存温度的影响。所有被检测的变量都对表观基因组图谱没有显著影响。在储存了 13 ~ 17 年的样本中，没有观察到储存时间的系统性影响。目前储存在生物库中的大多数样本只要满足本研究中定义的收集和储存标准，就可以进行有意义的组学分析。

17. **Pinto J, Domingues MR, Galhano E, et al. Human plasma stability during handling and storage: impact on NMR metabolomics[J]. Analyst, 2014, 139(5):1168-1177.**

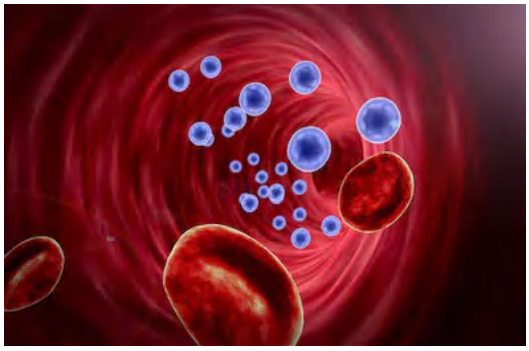
摘要

目前，人类血浆在处理和存储过程中的降解问题，是核磁共振（NMR）代谢组学最重要的问题，本研究工作有助于填补这方面的不足。肝素与 EDTA 抗凝管相比，前者不受多糖干扰，同时保留了全光谱信息。在时间/温度条件下，从 2.5 小时起，室温对脂蛋白和胆碱化合物有很大影响。此外，在-20℃下短期储存最长为 7 天，但在 1 个月内，建议使用-80℃存储。此外，在重复使用血浆样本的情况下，建议不超过 3 次连续反复冻融。最后，在调查了特定非禁食条件的可能混淆性后，在部分匹配的非禁食队列（n = 49）中，发现长期-80℃储存（长达 2.5 年）的影响几乎可以忽略不计。

18. **Suarez-Diez M, Adam J, Adamski J, et al. Plasma and Serum Metabolite Association Networks : Comparability within and between Studies Using NMR and MS Profiling[J]. J Proteome Res, 2017, 16(7):2547-2559.**

摘要

血液是代谢组学研究中最常用的生物流体之一，血清和血浆组分通常用作血液本身的替代品。在这里，我们研究了一系列 29 种代谢物的关联网络，这些代谢物通过核磁共振在两个队列的 1000 名健康献血者的血浆和血清样本中进行了鉴定和量化。第二次研究共 377 人，从同一个人身上提取血浆和血清样本，用 FIA-MS/MS 检测和定量 122 种代谢物，采用四种不同的推断算法（ARANCE、CLR、CORR 和 PCLRC）获得一致网络。不同研究所得的血浆和血清网络具有不同的拓扑性质，血清网络的连通性比血浆网络强。在全球范围内，从健康人的同一血液样本中获得的血浆和血清组分的代谢物关联网络显示出相似的拓扑结构，在局部范围内，会出现一些差异，如氨基酸的检测。



19. Yin P, Lehmann R, Xu G. Effects of pre-analytical processes on blood samples used in metabolomics studies[J]. Anal Bioanal Chem, 2015, 407(17): 4879-4892.

摘要

每天，分析化学家和生物分析化学家都在不断努力提高其方法的灵敏度、特异性、稳定性和重现性。特别是在有针对性和非有针对性的分析方法中，包括代谢组学分析，这些目标并不容易实现；然而，可靠和可重复的测量和低变异系数（CV）对于成功的代谢组学方法至关重要。然而，如果样品质量差，即在同事采集样本过程中出现分析前错误，那么分析师的所有努力都是徒劳的。分析前的风险和错误比预期的更常见，即使使用了标准操作程序（SOP）。这一风险在临床研究中特别高，样本质量差可能严重影响最终分析结果的变异系数，导致研究结果令人失望，最终，尽管不合理，使得提供样本者的采集方法成为分析性能的关键问题。本文综述了液相色谱-质谱联用驱动体液代谢组学分析的前分析阶段。本文将讨论几个重要的分析前因素，这些因素可能会严重影响所研究的体液代谢组的分布，包括样本采集前的因素、采血、全血的后续处理（运输）、血浆和血清的处理以及样本储存条件的不足。此外，还将详细描述对血液代谢组稳定性的潜在影响，并提出在分析前阶段规避风险的实用程序建议。

20. Paglia G,Del Greco FM, Sigurdsson BB, et al. Influence of collection tubes during quantitative targeted metabolomics studies in human blood samples[J].Clin Chim Acta,2018,486:320-328.

摘要

背景：血浆和血清是临床研究中使用最广泛的基质。但是，在采血管基质中可能会观察到代谢物绝对浓度的某些变化。

方法：我们使用相同的方案对来自南蒂罗尔合作卫生研究的 80 名受试者（40 位健康的老人和 40 位健康的年轻人）三种标本（血清、EDTA 血浆和柠檬酸盐血浆）的 189 种代谢物进行了定量靶向代谢组学分析（LC-MS/MS AbsoluteIDQ p180 Kit Biocrates）。

结果：血清中的浓度水平高于柠檬酸盐血浆和 EDTA 血浆，尤其是氨基酸和生物胺。然而，这两类代谢物的平均皮尔逊相关系数始终高于 0.7。我们还可以证明未采血的 EDTA 采血管中含有大量肌氨酸。最后，我们比较了年轻人和老年人的代谢组，发现在血清中检测到随着年龄增长而发生显著变化的代谢产物。

结论：血清标本为生物标志物发现研究提供了更高的敏感性。由于 EDTA 采血管中存在大量的肌氨酸， EDTA 血浆不适合肌氨酸的准确定量研究。

21. Lin Z, Zhang Z, Lu H, et al. Joint MS-based platforms for comprehensive comparison of rat plasma and serum metabolic profiling[J]. Biomed Chromatogr, 2014, 28(9): 1235-1245.

摘要

代谢组学是全面理解与药物、化学物质和环境引起的扰动相关细胞和机体特异性反应的快速发展领域。血液标本经常用于临床和生物学研究。在这项研究中，我们比较了使用气相色谱-质谱（GC-MS）和液相色谱-四倍飞行时间质谱（LC-QTOF-MS）的互补平台在大鼠血浆和血清之间的代谢谱。测试的样品类型包括用 EDTA.K_2 制备的血浆和血清管制备的血清标本。质量控制样品中每种检测到的代谢物/特征的峰面积变化结果表明，在 LC-QTOF-MS 中具有良好的重现性，在 GC-MS 中具有更好的重现性。在 GC-MS 分析中：（a）25.8%的定义代谢物血清与血浆谱分析存在差异（t 检验，$p < 0.05$）；（b）血清在代谢谱分析中通常具有较高的峰值强度，因此具有比血浆更高的敏感性。在 LC-QTOF-MS 分析中，鉴定出 13 种（正离子模式）和 7 种（负离子模式）重要的代谢产物。

22. Breier M, Wahl S, Prehn C, et al. Targeted metabolomics identifies reliable and stable metabolites in human serum and plasma samples[J]. PLoS ONE, 2014, 9(2): e89728.

摘要

个体中代谢物水平随时间变化的信息可以估计代谢物测量值的可重复性。在干预研究中，至关重要的是适当地判断由任何一种干预引起的变化。在多中心研究中，分析前阶段（采集、运输和样品处理）是数据质量特别重要的组成部分。使用靶向 LC-MS 方法分析了 22 位健康人的空腹血清和血浆样品中代谢物的可靠性（人与人之间的差异，类内相关系数）和稳定性（不同温度下运输模拟，分离胶采血管，冻融循环）。血清中代谢物测量的可靠性高于血浆样品，尤其是大多数饱和的短链和中链酰基肉碱、氨基酸、生物胺、甘油磷、鞘脂和己糖。大多数代谢产物在冷藏袋中和室温下，在非离心管中稳定 24 小时。血浆和血清代谢物的稳定性表现出良好的一致性。血清代谢物浓度大部分不受试管类型和一两个冻融周期的影响。假设单个时间点测量就足以对大多数代谢物进行目标代谢组学分析。理想情况下，样品应在采集后立即分离并冷冻，因为一些氨基酸和生物胺在冷藏袋中 3 小时内会变得不稳定。血清分离胶管可安全用于此过程，因为它们对大多数代谢物的浓度没有影响。对于大多数代谢物，将非离心样品装在冷袋中是一种经济高效的选择。

23. Yoko Fukugawa, Hiroaki Ohnishi, Takahiro Ishii et al. Effect of Carryover of Clot Activators on Coagulation Tests During Phlebotomy. Am J Clin Pathol 2012;137:900-903.

摘要

我们调查了血清管促凝管对主要凝血试验的影响。首先，将来自 30 名正常受试者的血液样本与少量含促凝剂的液体混合，并确定它们对各种凝血测试的影响。当将含凝血酶的液体添加到血液样本中时，只有纤维蛋白单体复合物的值显示出显著变化。随后，按照标准采血程序，分别在血清管之前和之后用血凝管采集 100 人的血液样本（分别来自 75 名健康志愿者和 25 名服用华法林的患者）。进行了各种凝血试验，以确定含凝血酶的血液污染对凝血参数的影响。两种采血管之间的差异很小，但对于某些凝血测试而言却很明显。因此，我们得出的结论是，当使用标准采血技术时，血清管中的促凝剂对凝血试验的影响极小。

24. Sampson M, Ruddel M, Albright S, Elin RJ. Positive interference in lithium determinations from clot activator in collection container. Clin Chem 1997;43:675-679.

用 10 mL 普通的红帽塑料 Vacutainer Plus 管（Becton Dickinson）采集血液标本，管内含有二氧化硅促凝剂和有机硅表面活性剂（产品编号：36-7820），本文研究了该管对锂离子选择电极测定的正干扰（Lytening 2Z 分析仪；Dade）。我们评估了原始试管（蓝色标记）和配制为包含更少的有机硅表面活性剂（条纹标记）的新试管。我们确定干扰是来自二氧化硅促凝剂或用于将二氧化硅固定到试管的硅氧烷表面活性剂，并且与试管中的血液量成反比。锂离子选择性电极长期间歇暴露于二氧化硅促凝剂或表面活性剂会导致锂离子值降低，这是因为二氧化硅促凝剂或表面活性剂的正干扰。此外，Dade

对照材料（QCLytes）未检测到对患者样品的锂离子选择电极的这种长期干扰。

25. **Steven C. Kazmierczak, Steven C. Kazmierczak, Steven C. Kazmierczak, et al. False-positive troponin I measured with the Abbott AxSYM attributed to fibrin interference. International Journal of Cardiology 101(2005)27-31.**

背景：血清通常用于测量心肌肌钙蛋白 I（cTnI）。先前的研究表明，血清样本中的纤维蛋白可能会干扰该 cTnI 的测量。我们使用 AxSYM 分析仪对 cTnI 结果增加的所有标本进行重复分析，调查了血清标本中纤维蛋白干扰的发生率和强度。

方法：在 4 个月的时间里，我们用雅培 AxSYM 分析了 3692 个样本的 cTnI。其中 307 例（8.3%）显示 cTnI 增加。阈值是方法精度的三倍（15%），用于判断样品重复分析之间的差异。在初始和重复 cTnI 分析之间重新离心所有样本。

结果：307 例患者的 cTnI 浓度升高，其中 24 例（7.8%）重复分析之间的差异大于 45%。最初分析这 24 个样品时得到的 cTnI 浓度范围为 2.4 ~ 24.0 mg/L。重复分析显示 20 个样品的重复值（83%）在正常参考区间内，而 16 个样品的重复值（67%）表示浓度低于 0.3 mg/L。

结论：我们的发现表明，雅培 AxSYM 最初测量 cTnI 浓度在 2.0 ~ 25.0 mg/L 范围内的血清样本中，应该高度怀疑干扰。在测得的肌钙蛋白浓度大于 25.0 mg/L 的标本中没有发现干扰，这表明血纤蛋白的干扰作用通常不足以使 cTnI 杂散升高到该范围。此外，选择血浆作为 AxSYM 的首选标本，对 200 多个初始 cTnI 浓度为 2.0 mg/L 或更高的患者样品进行重复分析后，我们没有观察到任何差异的 cTnI 结果。

26. **Simone Canovi, Daniele Campioli, Luigi Marcheselli. Specimen Recentrifugation and Elevated Troponin I Levels. Lab Med Winter 2015;46:10-50.**

目的：比较标本离心前后血浆中肌钙蛋白 I 的水平。

方法：在连续 24 天的时间里，我们最近收集了 189 个血浆样本中的肌钙蛋白 I 水平高于阳性截断值（60 ng/L），然后重新测量了肌钙蛋白 I。

结果：两尾 Wilcoxon 配对对测试结果确定了近期离心前后肌钙蛋白 I 浓度之间的统计学差异（$p < 0.01$）。对于接近临界值的 94 个样本（在 189 个样本的总样本中，第 50 个百分位数以下），Passing-Bablok 回归分析显示离心后肌钙蛋白 I 的浓度降低的中位数为 10 ng/L（95%置信区间为-37.5 ng/L ~ -10.0 ng/L）。

结论：标本重新离心后，心脏肌钙蛋白 I 升高的标本中的肌钙蛋白 I 浓度降低，存在患者分类错误的风险。而且，这种做法导致周转时间的不必要延长。

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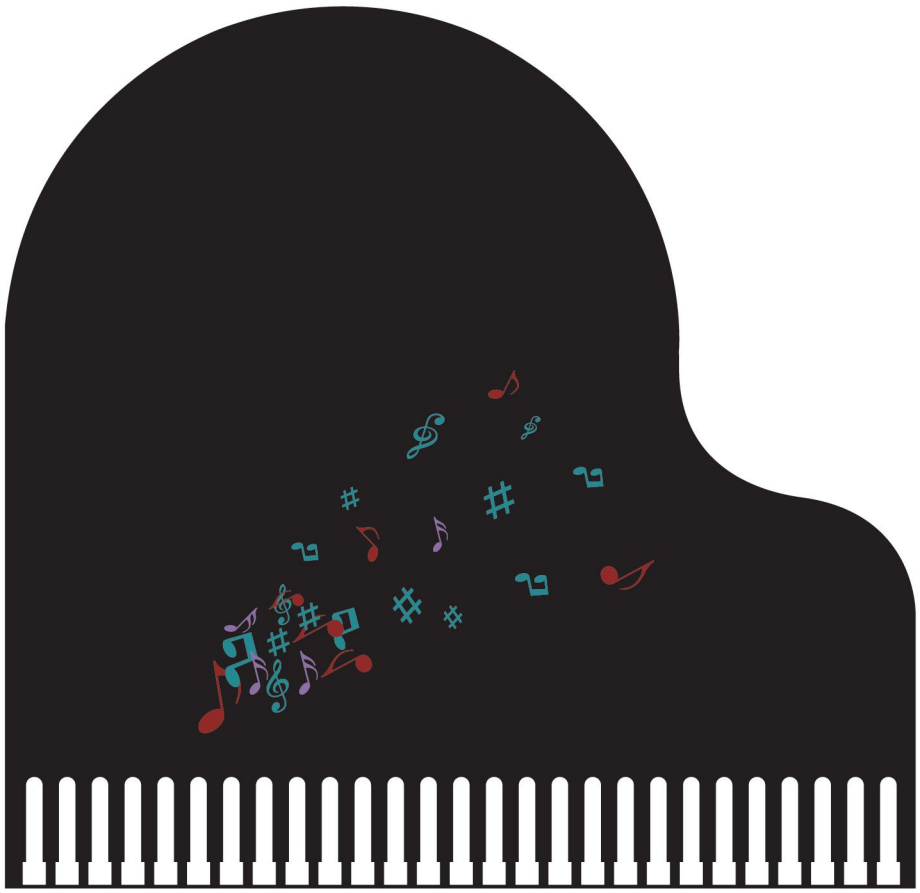

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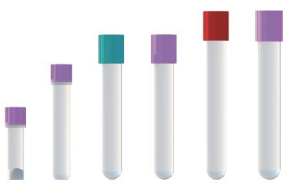

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Featured Article

文献精读

本期的文献精选是一篇研究文章《采血管对血液标本中定量靶向代谢组学研究的影响》，原文标题为《Influence of collection tubes during quantitative targeted metabolomics studies in human blood samples》。本文作者使用 BiocratesAbsoluteIDQ® p180 试剂盒和超高效液相色谱结合 Q-Trap 质谱仪对 189 种代谢物进行了定量靶向代谢组学分析，结果揭示血清管为生物标志物发现提供了更高的敏感性，肌氨酸的准确定量应避免使用 EDTA 管，柠檬酸盐管可能是脂质组学的更好选择。

采血管对血液标本中定量靶向代谢组学研究的影响

摘要

背景：血浆和血清是临床研究中使用最广泛的基质。但是，在采血管基质中可能会观察到代谢物绝对浓度的某些变化。

方法：我们使用相同的方案对来自南蒂罗尔合作卫生研究的 80 名受试者（40 位健康的老人和 40 位健康的年轻人）的三种标本（血清、EDTA 血浆和柠檬酸盐血浆）的 189 种代谢物进行了定量靶向代谢组学分析（LC-MS/MS AbsoluteIDQ p180 Kit Biocrates）。

结果：血清中的代谢产物浓度水平高于柠檬酸盐血浆和 EDTA 血浆，尤其是氨基酸和生物胺。然而，这两类代谢物的平均皮尔逊相关系数始终高于 0.7。我们还可以证明未采血的 EDTA 采血管中含有大量肌氨酸。最后，我们比较了年轻人和老年人的代谢组，发现在血清中检测到随着年龄增长而发生显著变化的代谢产物。

结论：血清标本为生物标志物发现研究提供了更高的敏感性。由于 EDTA 采血管中存在大量的肌氨酸，EDTA 血浆不适合肌氨酸的准确定量研究。

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关键字：靶向代谢组学；EDTA 血浆；血浆柠檬酸；血清；肌氨酸

缩写：LC-MS，液相色谱质谱法；CHRIS，南蒂罗尔合作卫生研究；FIA，流动注射分析；SOP，标准操作程序；UHPLC，超高效液相色谱；HILIC，亲水作用液相色谱；PCA，主成分分析；PC 1，第一主要组成部分；PC 2，第二主要组成部分；PC 4，第四主要组成部分；QC，质量控制；ADMA，不对称二甲基精氨酸；SDMA，对称二甲基精氨酸；C18:1，十八碳酰肉碱；PC aa C40:3，1,2-二酰基-sn-甘油-3-磷酸胆碱 40:3

1. 介绍

代谢组学是由生物系统中小分子的全面识别和定量驱动的新陈代谢系统级分析。代谢组学紧密结合了表型，因为它整合了个体的遗传背景、衰老和生活方式。代谢组学分析可以在不同的样本类型中进行，例如组织[1]、细胞[2-5]和生物流体[6]。近年来，生物库和高通量技术的同步发展已使新一代成功的大规

模队列研究得以应用，其中代谢组学可以应用[7-13]。

生物库为研究人员提供大量血液制品。血浆和血清这两种血液成分是临床研究中使用最广泛的基质，尽管血液采集还有其他选择，例如干血斑（DBS）[14]和微量采血（VAMS）[6]。无需经过培训的工作人员即可进行远程采集的机会。通过离心将血浆和血清从全血中分离出来。血清采血管中装有分离凝胶，凝血后会分离血液。另一方面，血浆采血管中含有抗凝剂，例如 EDTA、肝素或柠檬酸盐，可防止血液凝固。

选择正确的生化分析基质对于避免错误的诊断至关重要[15]。在临床研究的分析前阶段，采血管通常是一个未被充分认识的变量，因此在代谢组学分析中评估这些基质的影响非常重要[16-18]。

液相色谱质谱（LC-MS）是用于代谢组学研究的最受欢迎的平台之一。在文献中，有几份报告探讨了采血管在代谢分析研究中的影响，但几乎所有报告都比较了非靶向数据而非定量数据[19-21]。

使用市售试剂盒进行定量靶向代谢组学研究已变得很流行，因为它简化了实验室间的比较以及来自不同研究的数据交换[22]。在这些试剂盒中，可用于多种 LC-MS/MS 仪器的 BiocratesAbsoluteIDQ®p180 试剂盒已应用于许多血清和血浆研究，包括几项大规模的前瞻性队列研究[7-9,11-13,23]。最近的研究使用相似的市售试剂盒（BiocratesAbsoluteIDQ®p150）比较了 EDTA 血浆和血清对靶向代谢组学的影响[24]。

本研究的目的是调查在南蒂罗尔合作健康研究（CHRIS）样本中采血管对定量靶向代谢组学研究的影响，以确定哪种采血管更适合大规模代谢组学的队列研究。CHRIS 是一项基于人群的研究，研究了常见慢性病的遗传和分子基础及其与普通人群的生活方式和环境的相互作用。从参与者采集了一些生物标本，包括血清、EDTA 血浆和柠檬酸盐血浆，并保存在 CHRIS 生物库中[25]。

为此，我们从 CHRIS 队列中选择了 80 名看起来健康的受试者。尚不知道这 80 名患者是否患有任何生理或精神疾病，并使用 Absolute IDQTM p180 试剂盒进行了分析，从而测量了三种所研究基质中的 189 种代谢物。

2. 材料和方法

2.1 研究设计和标本采集

CHRIS 研究已在前面进行了详细描述[25]。简而言之，这项研究是在意大利南蒂罗尔的维诺斯塔山谷进行的。招聘工作逐步进行，选定研究区域中所有 28000 名成年人都应邀参加，每个工作日最多 10 人。

在这项研究中，我们纳入了从 2011 年 8 月 24 日至 2014 年 7 月 15 日参加研究的首批 4979 名参与者中的 80 名参与者。

共有 40 名年龄小于 35 岁的独立个体被选入“年轻”组。选择其他 40 名独立受试者，年龄大于 60 岁，并分配到“老年人”组。所有选定的个体均无亲缘关系，没有任何被诊断出的心血管、代谢或其他慢性疾病，也没有定期服用任何药物。两组均性别匹配。另外，为了排除任何可能影响季节的因素，选择需要参加的个人可从同一季节开始[26]。

过夜禁食后，在募集中心采集血液，并在很短的时间间隔内清晨采血。我们遵循世界卫生组织关于在实验室诊断性研究中使用抗凝剂以及血液、血浆和血清标本稳定性的指南（WHO/DIL/LAB/99.1 Rev.2，网址为 http://apps.who.int/iris/handle/10665/65957）。为了确保测量的可靠性和准确性，受过训练的护士在抽血后立即进行了分析前处理。血清管、EDTA 管和柠檬酸盐管由 Greiner 提供。采血后立即将所有真空采血管颠倒五次，以将抗凝剂或促凝剂与全血混合。

将血清管在室温下凝固 30 分钟，并在室温下以 1500 × g 离心 15 分钟。EDTA 管和柠檬酸盐管采血后，室温下在标本混匀仪混匀 10 分钟。EDTA 管未做进一步处理，柠檬酸盐管室温下以 1500 × g 离心 15 分钟。

将同一天的 EDTA 管、柠檬酸盐管和血清管一起保存在室温的运输袋中，并运送到实验室/生物库。在整个招募期间，固定并监控了运输过程中的温度范围。血清、EDTA 血浆和柠檬酸盐血浆等分为 120 µL 的标本，并在 Bozen/Bolzano 生物库中-80℃保存。

所有参与者均根据赫尔辛基宣言签署了知情同意书。该研究获得了博尔扎诺自治省医疗保健系统伦理委员会的伦理批准。

2.2 化学试剂

乙腈购自美国 VWR，甲醇购自德国 Sigma-Aldrich。用配备有 LC-pak®抛光机（美国密理博）的 Milli-Q 净水系统制备水。所有溶剂均为 LCMS 分析纯或更高纯度。NISTEDTA 血浆 质控样本和 1950 标准参考材料购自 NIST（美国马里兰州盖瑟斯堡）。

2.3 液相色谱质谱

使用 BiocratesAbsoluteIDQ®p180 试剂盒和超高效液相色

谱（UHPLC）（安捷伦 Agilent 1290），结合 Q-Trap 质谱仪（MS）（QTRAP 6500，Sciex）进行靶向分析。

根据制造商的操作程序进行样品制备和分析。简而言之，样品处理程序是在单个样品上进行的，并采用 96 孔板设计，其中样品进行了衍生化和分析物提取。该试剂盒需要 10 μL 样品，并提供基于人体血浆的 3 种浓度水平（低、中、高）的质量控制，可用于质量控制和批次归一化。一旦处理完，每个样品都要进行两次单独的基于 MS 的分析。试剂盒中总共包括 189 种代谢物，通过 UHPLC-MS/MS 测定了 43 种代谢物，通过流动注射分析（FIA）-MS/MS 测定了 146 种代谢物。同位素标记的和化学同源的内标用于定量，总共提供了 56 种被完全确认为绝对定量的分析物。UHPLC-MS/MS 运行通过充分验证的 43 种代谢物的绝对定量，包括 21 种氨基酸和 22 种生物胺，并使用了 7 种不同浓度的外部校准标样和同位素标记的大多数分析物内标。剩余的 146 种代谢物，包括 40 种酰基肉碱、90 种甘油磷脂、15 种鞘脂和 1 种己糖，通过 FIA-MS/MS 进行分析，使用具有代表性内部标准的一点内标校准（9 种同位素标记的酰基肉碱、1 种同位素标记的己糖、1 个未标记的 lyso-PC、2 个未标记的 PC、1 个未标记的 SM，共 14 个内标）。通过 FIA-MS/MS 分析的 12 种酰基肉碱和己糖总和已完全定量，而由于缺少市售的专门内部标准，该试剂盒提供了“半定量”测量。本试剂盒中分析的几种脂质代表了可能的等压线和结构异构体的总浓度。

通过基于 MS 的无针对性工作流程运行 vacutainer 无添加剂血清管、柠檬酸盐血浆管和 EDTA 血浆管，可以实现肌氨酸确认[6]。简而言之，将 500 μL 水：甲醇（1.1）添加到每支采血管中，4000 RPM 涡旋 5 分钟，4300 RPM 离心 20 分钟，然后转移到新管中。将样品放入真空蒸发器（EZ-2，Genevac，Ipswich，英国）中，35℃真空干燥 120 分钟，然后用 200 μL 乙腈：水（50：50，v：v）复溶。

通过与 Q-TOF 质谱仪（MS）（Triple TOF 5600+，Sciex）结合的超高效液相色谱（UHPLC）（Agilent 1290）分析了肌氨酸确认的样品。色谱分离基于亲水相互作用液相色谱（HILIC），并使用 Acquity BEH 酰胺，100 × 2.1 mm 色谱柱（Waters Corporation）。

使用乙腈 + 0.1%甲酸作为流动相 A，水 + 0.1%甲酸作为流动相 B 进行分离。进样量为 5 μL，流速为 0.6 mL/min。使用以下线性梯度：0 分钟 95% A，1 分钟 95% A；4 分钟 30% A；5 分钟 30% A；5.1 分 95% A；8 分钟 95% A。

质谱仪在 50 ~ 1000 m/z 的质量范围内以全扫描模式运行，累积时间为 250 毫秒。在 ESI+模式下，离子源温度设定为 700℃、消簇电位为 30 V、碰撞能量为 6 V、离子喷雾电压为 5120 V、帘气为 25 psi，离子源气体 1 和 2 为 60 磅/平方英寸在 ESI 模式下，离子源温度设定为 650℃、消簇电位为-45 V、碰撞能量为-6 V、离子喷雾电压为-3800 V、幕气 25 psi、离子源气体 1 和 2 在 30 psi 下。通过自动校准对仪器进行质量校准，将 Sciex 阳性校准溶液（零件号 4460131， Sciex）注入正模式，将 Sciex 阴性校准溶液（零件号 4460134，Sciex）注入负模式。每两次进样后进入模式。

2.4 统计分析

在标准化之前排除所有批次中缺失值均高于 80%的代谢物。使用以下方程[22]，仅对 FIA-MS 数据（酰基肉碱、甘油磷脂、糖脂和己糖）进行批间标归一化：

归一化 Xij = Xij * [参考批次中参考质量控制的平均值（代谢物 i）/参考质量控制中的平均值（代谢物 i）]

作为参考批次，我们选择含有血清样品的批次，因为它的缺失值数量最少。制造商的质量控制（QC）“中等”——由掺有 59 种代谢物的人血浆混合物组成的 QC2 被用作参考质量控制。

归一化后，质控样品中具有变异系数（CV 高于 30%）的代谢物被排除在外。这项研究的最终数据集由 138 种代谢物组成。

管的差异是采用经典的统计方法进行评估的。用相应的检出限（在半定量代谢物中）或定量（在定量代谢物中）代替缺失的浓度后，进行了主成分分析（PCA），在无假设的情况下捕获采血管之间的变化和样品中的结构变异。随后，我们使用每个单一代谢物的方差分析（单向方差分析）研究了管依赖性

丰度变化。

对数转化浓度进行 PCA 分析。因此，假设集中浓度，我们通过奇异值分解方法将它们分解为潜在结构（PCs）。我们探索了可解释原始代谢产物总变异约 70%的 PCs。

在单向方差分析中，我们使用代谢物的对数变换测试了采血管之间的显著差异，以确保使用图形方法检查的正态性假设。我们以图形方式和通过 Barrlett 检验研究了方差的均匀性。我们应用了保守的 Bonferroni 校正法，对 138 种经测试的代谢物进行了计算（$p = 3.6 \times 10^{-4}$）。

此外，使用 CV 探索了每种代谢物浓度中自然发生的生物学变异性，以确定用于量化代谢物的方法是否稳定，或者是否随采血管的变化而变化。

为了描述数据，探索了采血管之间的成对相关性。在排除至少一支采血管中缺失值的受试者后，针对每种代谢产物计算皮尔逊相关系数（r），以比较血清与 EDTA 血浆，血清与柠檬酸盐血浆以及 EDTA 血浆与柠檬酸盐血浆。显示至少一个 r < 0.4 的代谢物被排除在进一步的数据分析之外。对数转换后的代谢物丰度进行 t 检验以比较老年人和年轻人的浓度，显著性水平为 3.6×10^{-4}。

所有统计分析都是使用 R 软件包版本 3.4.2（www.r-project.org/）和 Rstudio 版本 1.0.143 接口（http://www.rstudio.com/）进行的。使用 prcomp 函数安装 PCA。使用 R 和 MetaboAnalyst [27]绘制图表。

3. 结果

这项研究的目的是评估用于采血管对代谢产物定量靶向分析的影响。最终目标是确定哪种采血管更适合大规模代谢组学研究。

因此，我们设计了一个实验，旨在比较来自三种不同采血管中的 CHRIS 队列[25]血样，从而得出来自同一受试者的三个独立样本，分别是血清、EDTA 血浆和柠檬酸盐血浆。我们从 CHRIS 队列中选择了 80 名明显健康的受试者，40 名 35 岁以下的个体和 40 名 60 岁以上的个体。

我们使用针对目标代谢组学（Absolute IDQTM p180 试剂盒）的相同方案分析了所有样品，从而通过 LC 或 FIA 联用串联质谱（MS/MS）测量了 189 种代谢物。

3.1 预处理和规范化

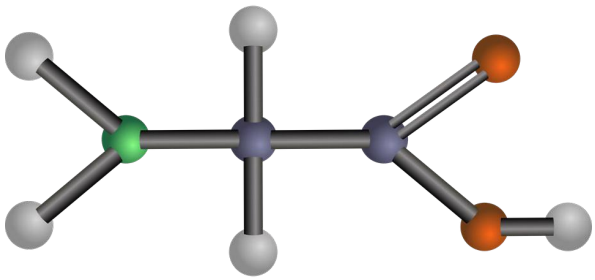
以三个分析批次分析样品。每批样品设计为包含 80 个样品、7 个校正剂、试剂盒供应商提供的 3 个血浆质量控制（QC）样品、1 个代表 CHRIS 队列的内部血清 QC 样品和 1 个商业血浆 NIST QC 样品。作为试剂盒一部分提供的 QC 样品由掺有 3 种标称浓度（“低”——QC1，“中”——QC2，“高”——QC3）的 59 种代谢物的人血浆混合物组成（分别由 LC 和 FIA 测得 42 和 17 种）。第二个 QC 样品是常规用作 QC（NIST SRM 1950）的参考血浆样品[28]。第三个质量控制由来自 CHRIS 队列的内部血清样本库组成[25]。来自同一采血管的样品在同一分析批次中以随机顺序进行分析。

在所有三种采血管中，我们检测到了相似数量的缺失值。在柠檬酸盐血浆中，我们检测到丢失值的 30.8%，在 EDTA 血浆中检测到 30.9%，在血清中检测到 30.1%。

接下来，我们考虑了质量控制（QC）样品的可重复性，以区分可能影响我们发现的潜在偏差和批次影响。三批中所有 QC 的 CV%显示：只有 37%的目标代谢物 CV%低于 10%（图 S1）。值得注意的是，通过 LC-MS 和 FIA-MS 分析的样品中 QC 的行为非常不同。QC 样品中的氨基酸和生物胺（通过 LC-MS 分析）分别显示 97%和 77%代谢物的 CV%低于 10%（图 S2），而 FIA 中分析的代谢物显示出更高的变异性，尤其是如图 S2 中所报道的磷脂和鞘脂的含量。这些结果与 Siskos 等人[22]先前的报告一致，发现使用相似的 AbsoluteIDQ®p150 Kit 采集的目标代谢组学数据在实验室间进行比较需要对 FIA-MS 中分析的样品进行标准化，而对 LC-MS 中分析的样品则不需要进行标准化。

因此，我们采用了与以前报道的相同的归一化策略[22]，并在材料和方法中进行了描述，其中使用血清作为参考批次，并使用制造商提供的质量控制样品（中度-QC2）作为参考质量控制，因为它记录了最少的缺失。

归一化后，氨基酸和生物胺（LC-MS）的 CV%并未提高（图 S1 和图 S2），相反，在 FIA-MS 中获得的代谢产物（甘油-磷脂、鞘脂和酰基肉碱）取得了很大的进步。这些结果证实，仅对在 FIA-MS 中获得的数据进行归一化是必要的[22]，因此仅对在 FIA-MS 中获得的数据应用了归一化。



3.2 血清、EDTA 血浆和柠檬酸血浆的比较

接下来，我们评估了采血管对目标代谢组学的影响。我们首先在数据集上应用主成分分析（PCA）。前两个成分（PC 1 和 PC 2）分别占数据集总方差的 48.9%和 9.3%，并且由于使用不同的采血管进行采样而没有显示任何效果（图 1a）。但是，探索其他主要成分后发现，第四种成分（PC 4）占数据集总方差的 5.5%，并将血清样品与柠檬酸盐血浆和 EDTA 血浆样品分开（图 1b）。因此，采血管也能解释数据集的某些差异，这表明血清中的代谢物与柠檬酸盐血浆和 EDTA 血浆获得的代谢物有所不同。

而且，柠檬酸盐血浆管中的样品似乎更紧密地聚集，表明较低的生物学差异（图 1）。为了证实这一点，我们比较了本研究中 80 名受试者在每个采血管中获得的受试者间差异。我们绘制了每种代谢物的几何平均值相对于 CV 的对数值，发现 EDTA 血浆和血清样品具有非常相似的生物学变化，但没有柠檬酸盐血浆（图 2）。总体而言，考虑到 80 名受试者中 75%的代谢物 CV 低于 30%，受试者之间的柠檬酸盐血浆变异性较低。另一方面，40%EDTA 血浆样品和 36%血清的代谢产物 CV 低于 30%（图 S3）。尽管三种采血管中氨基酸的生物学差异相似，但柠檬酸盐血浆中脂质（甘油磷脂和鞘脂）的受试者间变异性要小得多（图 2）。对于甘油磷脂，受试者之间的柠檬酸盐血浆变异性导致 68%代谢物的 CV 低于 30%，而 EDTA 样品中所有代谢物的 25%和血清中 19%的代谢物 CV 低于 30%。对于鞘脂，EDTA 样品中 93%代谢物的 CV 低于 30%，而 EDTA 样品中所有代谢物的 7%和血清中 20%的代谢物 CV 都低于 30%（图 2）。

但是，这些生物学差异不能解释 PCA 期间在第四部分中观察到的簇（图 1b）。为了更好地了解导致血清样品聚集的原因以及哪些代谢物对其起作用，我们进行了相关分析，并计算了三种不同采血管中每种单独代谢物的相对浓度差异。

作为一般趋势，我们观察到血清中的代谢物水平高于柠檬酸盐血浆和 EDTA 血浆中的代谢物（图 3）。特别是对于氨基酸和生物胺，我们在血清中记录到更高的浓度。对于氨基酸，相对差异的平均值在血清中比 EDTA 血浆高 7%，在血清中比柠檬酸盐血浆高 6%。对于生物胺，血清中相对差异的平均值比 EDTA 血清高 11%，血清比柠檬酸盐血浆高 15%。此外，生物胺和氨基酸的平均皮尔逊相关系数（R）始终高于 0.7（表 S1）。

更具体地说，我们发现在不同的采血管中有 17 种代谢物发生了显著变化（通过 Bonferroni 校正的 Anova 试验），其中 9 种氨基酸、6 种生物胺、1 种甘油-磷脂和 1 种酰基肉碱（表 1、图 3、图 4、图 S4、S5 和 S6）。仅在血清中检测到天冬氨酸和 5-羟色胺，而精氨酸和牛磺酸的相对差异最大。发现精氨酸在血清中比 EDTA 血浆中高 27%，在血清中比柠檬酸盐血浆中高 26%，而牛磺酸在血清中比 EDTA 血浆中高 39%，在血清中比柠檬酸盐血浆中高 38%（表 1、图 3 和图 4）。谷氨酰胺、丝氨酸、组氨酸、赖氨酸、苯丙氨酸、蛋氨酸和犬尿氨酸在血清中也显示出比柠檬酸盐血浆和 EDTA 血浆高的值（表 1、图 4 和图 S4、S5 和 S6）。血清和柠檬酸盐血浆中的 ADMA、SDMA 和谷氨酸水平相当，但 EDTA 血浆中较低，而柠檬酸血浆中的酰基肉碱 C6（C4.1-DC）水平高于血清和 EDTA 血浆（表 1、图 4 和图 S4、S5 和 S6）。

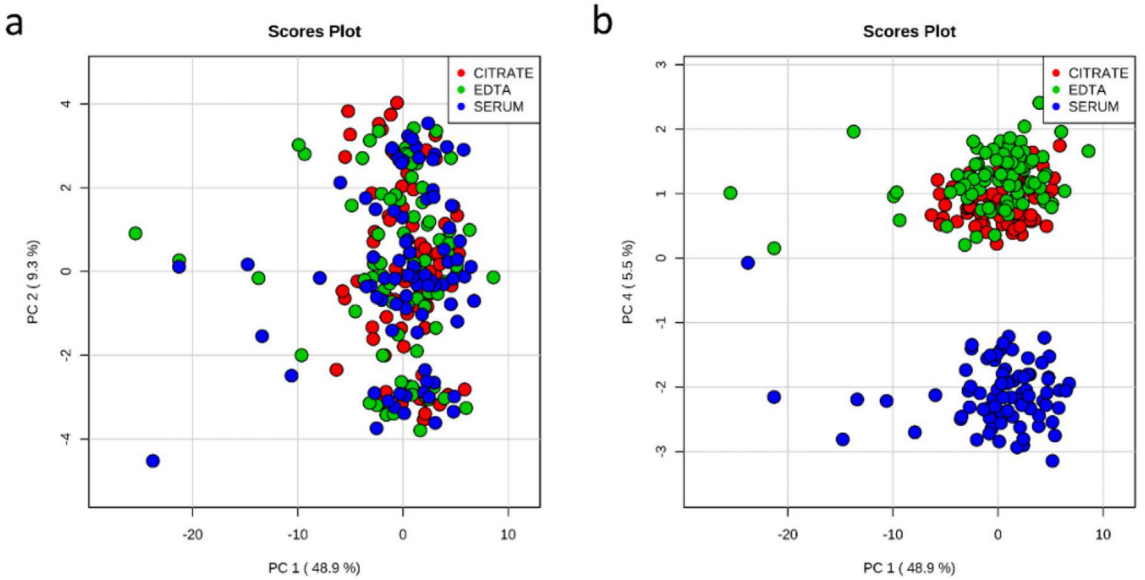


图 1. 主成分分析（PCA）。对从相同的 80 名参与者采集的血清、EDTA 血浆和柠檬酸盐血浆的靶向代谢组学数据进行 PCA。a）第二主成分（PC 2）与第一主成分（PC 2）的关系图。b）绘制第四主成分（PC 4）相对于第一主成分（PC 1）的图。

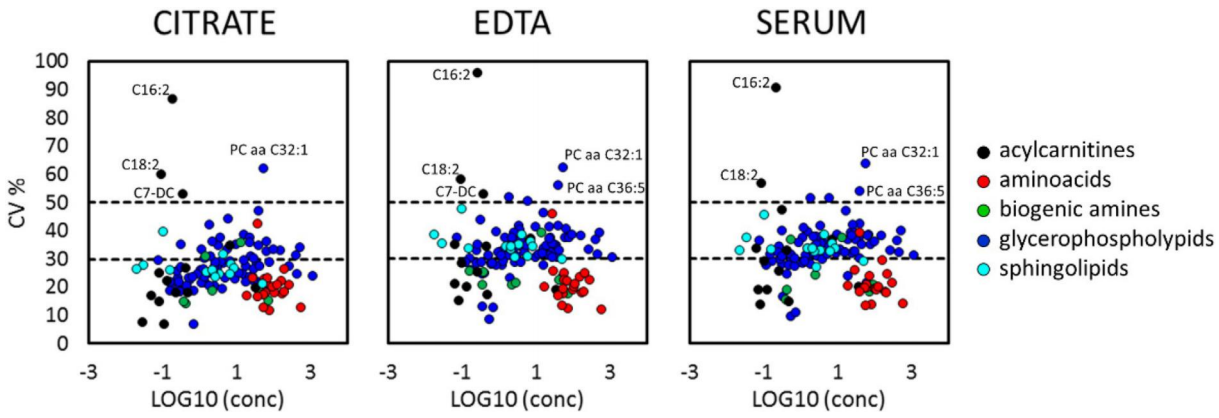


图 2. 生物变异。通过三种不同采血管中分析的每种代谢物百分比（CV%）随浓度［log_{10}（几何平均值）］变化的系数来衡量受试者间变异性的差异。

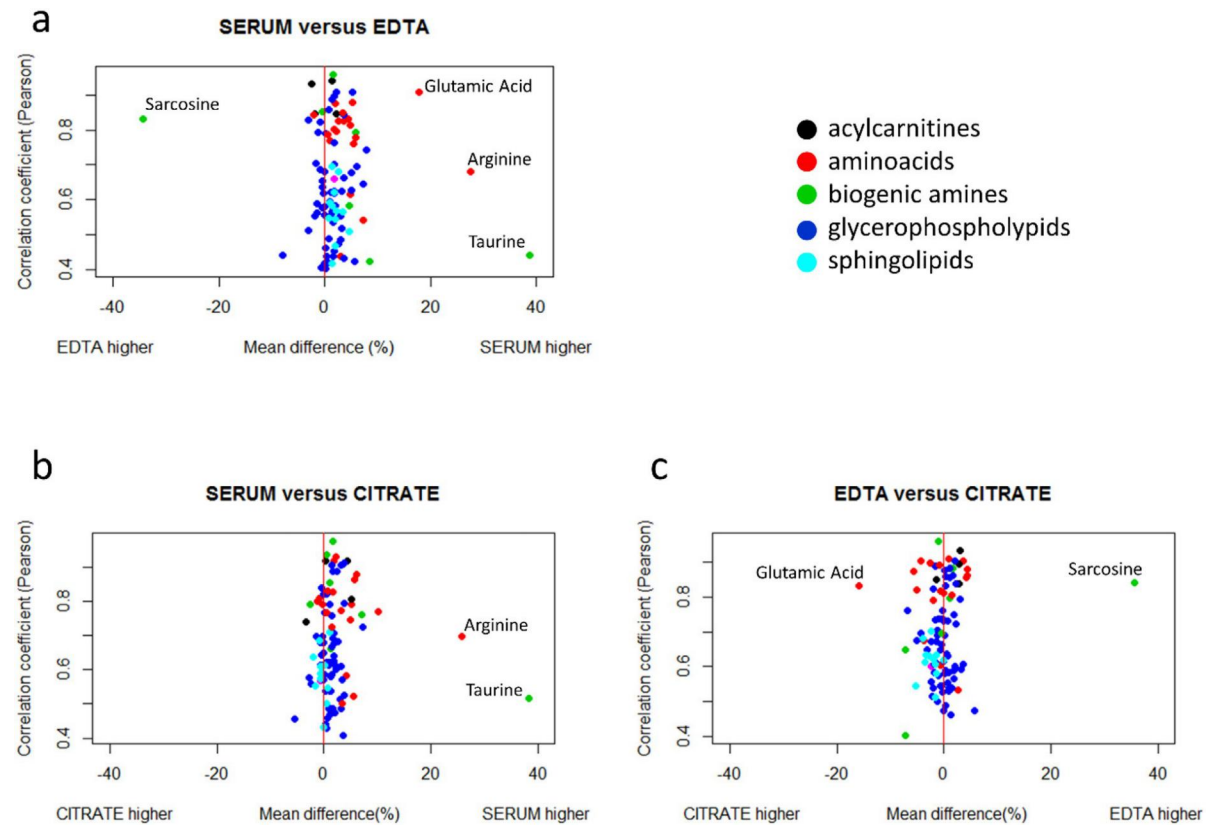


图 3. 相对浓度差和相关系数。x 轴表示相对浓度差的平均值，y 轴表示每种代谢物的相关系数。a）通过比较血清和 EDTA 血浆样品来计算相对浓度差异和相关系数。b）计算血清和柠檬酸盐血浆样品的相对浓度差异和相关系数。c）通过比较 EDTA 血浆和柠檬酸盐血浆样品，计算出相对浓度差和相关系数。

在 EDTA 血浆中发现仅有的浓度较高的两种代谢物是肌氨酸和 PC ae（42：3），尤其是肌氨酸在 EDTA 血浆中比血清高 34%，在 EDTA 血浆中比柠檬酸盐血浆高 36%（表 1、图 3 和图 4）。我们使用高分辨率质谱仪分析了血清管、EDTA 血浆管和柠檬酸盐血浆管的空白管含量。令人惊讶的是，我们发现空白的 EDTA 管中含有大量的肌氨酸，并使用精确的质谱和串联质谱法确认了该分子的身份（图 5）。在空白血清管和柠檬酸盐血浆管中未检测到肌氨酸（图 5）。在空白管中，没有发现其他在基质之间发生显著变化的代谢物（表 1）。

表 1. 不同采血管之间的代谢产物发生变化（采用 Bonferroni 校正的 ANOVA 测试）

种类	代谢物	ANOVA	血清-EDTA 血浆		血清-柠檬酸盐血浆		EDTA 血浆-柠檬酸盐血浆	
		p-Value	r	Mean diff%	r	Mean diff%	r	Mean diff%
氨基酸	精氨酸	1.1E-42	0.7	28	0.7	26	0.8	-2
氨基酸	赖氨酸	4.3E-04	0.8	5	0.8	5	0.7	0
氨基酸	组氨酸	1.0E-05	0.6	5	0.6	4	0.6	-1
氨基酸	苯丙氨酸	6.6E-10	0.5	7	0.5	3	0.7	-4
氨基酸	谷氨酰胺	1.4E-06	0.4	3	0.5	6	0.5	3
氨基酸	蛋氨酸	1.2E-06	0.4	8	0.4	3	0.4	-5
氨基酸	丝氨酸	9.5E-09	0.8	6	0.8	10	0.9	4
氨基酸	谷氨酸	2.7E-09	0.9	18	0.9	2	0.8	-16
氨基酸	天冬氨酸	2.5E-103	NA		NA		NA	
生物胺	尿嘧啶	1.7E-04	0.8	6	0.8	7	0.8	1
生物胺 s	牛磺酸	5.2E-79	0.4	39	0.5	38	0.7	0
生物胺	SDMA	1.8E-05	0.6	5	0.8	-3	0.7	-7
生物胺	血清素	7.6E-88	NA		NA		NA	
生物胺	ADMA	2.1E-09	0.4	9	0.7	1	0.4	-7
生物胺	肌氨酸	1.5E-56	0.8	-34	1.0	2	0.8	36
酰基肉碱	C6(C4:1-DC)	2.1E-07	NA		NA		NA	
甘油磷脂	PC ae(42:3)	3.8E-06	0.4	-9	0.4	1	0.5	10

缩写：r，皮尔逊相关系数。

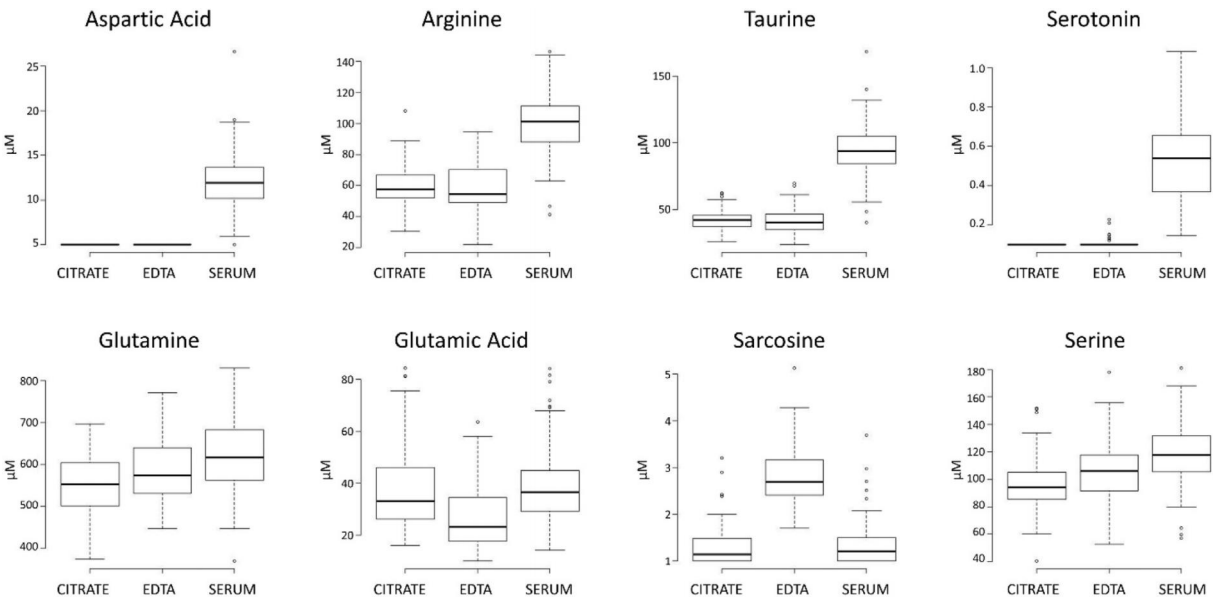


图 4. 不同采血管的代谢物水平。选定代谢物在血清、EDTA 血浆和柠檬酸盐血浆之间变化的箱线图。

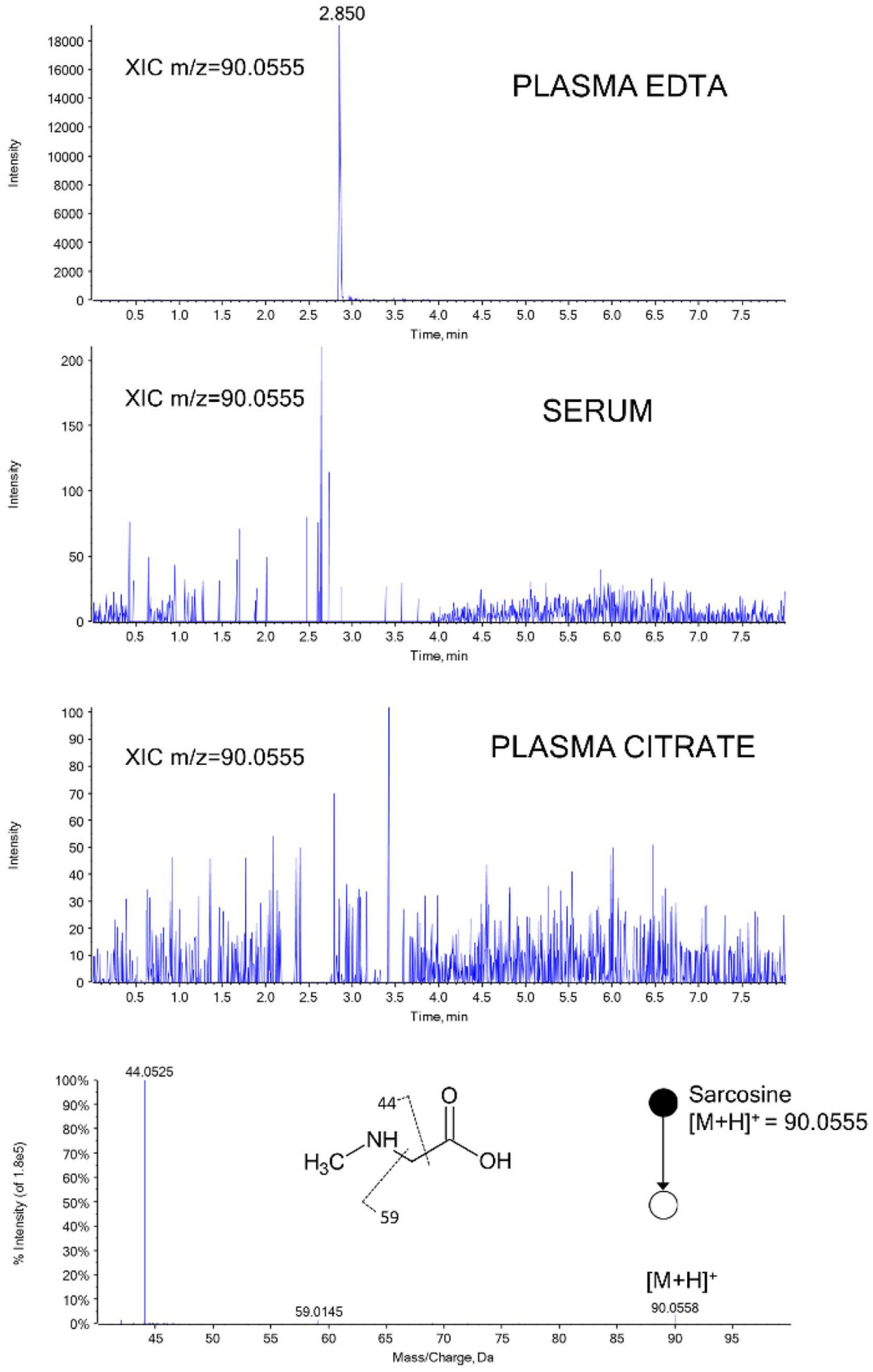


图 5. EDTA 管中存在大量的肌氨酸。通过串联质谱法和 EDTA 血浆管中的准确质量检测证实了肌氨酸。

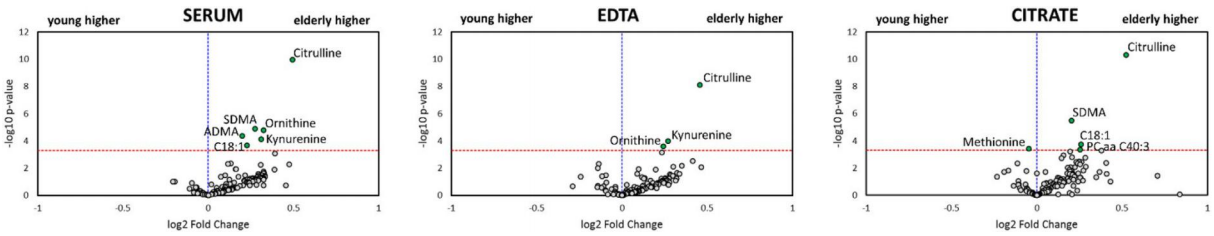


图 6. 年龄分布图。比较 40 个看起来健康的年轻人与 40 个看起来健康的老年人在不同采血管中的代谢物。

最后，为了研究不同采血管在分析大型队列时的影响，我们将看起来健康的年轻人代谢组与看起来健康的老年受试者代谢组进行了比较。使用单变量统计数据（采用 Bonferroni 校正的 t 检验），我们发现血清中检测到的代谢物数量随年龄的变化而显著变化。实际上，在老年受试者的血清中观察到 6 种代谢产物较高，即瓜氨酸、鸟氨酸、ADMA、SDMA、犬尿氨酸和 C18：1（图 6）。相同的分析在 EDTA 血浆中观察到瓜氨酸、鸟氨酸、犬尿氨酸三种代谢物发生变化（图 6）。在柠檬酸盐血浆中，代谢物发生了五种变化。老年受试者中瓜氨酸、SDMA、C18:1 和 PCaa C40:3 升高，而年轻受试者中的蛋氨酸则更高（图 6）。

4. 讨论

标准化是任何组学技术中的关键方面。在临床代谢组学中必须统一程序以确保分析前和分析的鲁棒性[29]。使用市售的定量靶向代谢组学试剂盒有助于简化临床代谢组学，因为它简化了实验室间的比较，并简化了来自不同研究的数据交换[21]。尽管如此，考虑到大多数临床代谢组学研究都包括来自不同血样，EDTA 血浆、柠檬酸盐血浆和血清的数据，因此选择合适的基质对于确保正确的临床结果非常重要。

因此，本研究探索了不同采血管对 CHRIS 生物库中人体血液样品代谢组的影响[25]。在这项研究中，我们没有评估血浆肝素管作为靶向代谢组学的基质，因为该血液样本未包括在 CHRIS 队列中。最近对血浆肝素管进行了非靶向代谢组学[21]和脂质组学[19]的评估，证明该采血管可能适合代谢组学的应用。

通常，我们观察到血清样品中代谢物含量比柠檬酸盐血浆和 EDTA 血浆样品中高，对于几种氨基酸和生物胺尤其如此，其中一些代谢产物在血清中的浓度比 EDTA 血浆中高，例如精氨酸[24,30]以及 ADMA 和 SDMA[30]，但这些研究未包括与柠檬酸血浆的比较。此外，我们的分析还确定了血清中比 EDTA 血浆和柠檬酸盐血浆高得多的其他代谢物，例如天冬氨酸、犬尿氨酸、牛磺酸和 5-羟色胺。

血清样品中较高水平的代谢物可能是由两个因素共同作用造成的。首先，在血清标本中，在标本采集和预分析过程中去除了一部分蛋白质，导致总体积较小，因此代谢产物更加浓缩。其次，在血清样品凝血过程中，某些代谢物可能会从血细胞中释放出来，特别是从活化的血小板中释放出来，从而增加其浓度[30,31]。

尽管如此，较高的代谢物水平也可能是由于生物学差异所致。实际上，矩阵之间的相关性分析提供了生物胺和氨基酸的高皮尔逊相关系数（R），这表明采血管之间代谢物水平的差异主要归因于所有个体之间的系统性变化。

血清样品还显示出更高的生物学差异，考虑到上述问题，这可能会得到部分解释（更复杂的采集程序需要凝血时间，并且可能在凝血过程中释放出可变数量的某些代谢物）。然而，在 EDTA 血浆管中发现了类似的生物学变异，但在柠檬酸盐血浆管中未发现。因此，我们的结果表明，采血管之间的背景噪声和分析前程序的组合可能会影响测量的准确性，尤其是对于血液中低浓度存在的代谢物而言。在这三种基质中，柠檬酸盐血浆样品提供了最低的生物学变异，尤其是脂质，这表明该基质可能是脂质组学的更好选择。

有趣的是，与本研究中分析的所有其他代谢物相比，肌氨酸的行为不同。确实，EDTA 血浆中的肌氨酸含量显著高于血清和柠檬酸盐血浆，这表明用于样品采集的 EDTA 管中存在大量肌氨酸。我们通过分析用于采集血清、EDTA 血浆和柠檬酸盐血浆样品的空白管含量，证实了肌氨酸仅存在于空白 EDTA 管中，从而验证了这一假设。由于肌氨酸最近已被确定为浸润性前列腺癌的潜在生物标志物，因此这尤其令人震惊[32]。我们的发现表明，由于真空容器 EDTA 管中存在大量的肌氨酸，因此在以后的研究中应避免使用 EDTA 血浆样品进行肌氨酸的准确定量。

考虑到血清中代谢物浓度的较高水平（以及较高的生物学差异），该基质可能为大型代谢组学研究中的生物标志物发现研究提供更高的敏感性。作为原理的证明，我们将看起来健康的年轻人代谢组与看起来健康的老年受试者代谢组进行了比较。我们的研究结果证实，血清中代谢物的数量随年龄的增长

而显著变化。

值得注意的是，最近有一些代谢物，例如瓜氨酸被用作衰老的生物标志物[33,34]，但是由于样本量有限，这些结果需要在更大的队列中重复使用。

综上所述，这些结果表明，可能是由于检测到的较高浓度，血清是生物标志物发现研究的较好基质。

5. 结论

在这项研究中，我们评估了定量靶向代谢组学过程中采血管对人体血液样本的影响。作为一般趋势，我们观察到血清中的代谢物浓度高于柠檬酸盐血浆和 EDTA 血浆，但考虑到大多数代谢物在采血管之间显示出高度相关性，因此选择用于定量代谢组学分析的血清管、EDTA 血浆管或柠檬酸盐血浆管确实在流行病学研究中不一定会引入明显的偏倚。然而，由于检测到更高的浓度，血清为生物标志物发现研究提供了更高的敏感性。有趣的是，柠檬酸盐血浆样品在 CHRIS 采集中为脂质提供了最低的生物学变异，表明该基质更适合于脂质的定量靶向代谢组学。最后，EDTA 血浆样品中浓度最低，其生物学变化与血清样品相似。此外，还发现 Vacuette® EDTA 试管中含有大量的肌氨酸，这表明该基质不适用于需要精确定量肌氨酸的研究。

最后，与显然健康的年轻受试者相比，在看起来健康的老年受试者中瓜氨酸、SDMA、C18:1 和 PCaa C40:3 有所增加，鼓励在更大的人群中发现更多的老化生物标志物。

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数据陈述

该数据集是 CHRIS 研究的一部分[25]，可应要求提供。

Influence of collection tubes during quantitative targeted metabolomics studies in human blood samples

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ABSTRACT

Background: Plasma and serum are the most widely used matrices in clinical studies. However, some variability in absolute concentrations of metabolites are likely to be observed in these collection tubes matrices.
Methods: We analyzed 189 metabolites using the same protocol for quantitative targeted metabolomics (LC-MS/MS AbsoluteIDQ p180 Kit Biocrates) in three types of samples, serum, plasma EDTA and citrate, of 80 subjects from the Cooperative Health Research In South Tyrol cohort (40 healthy elderly and 40 healthy young).
Results: The concentration levels were higher in serum than citrate and EDTA, in particular for amino acids and biogenic amines. The average Pearson's correlation coefficients were however always higher than 0.7 for these two classes of metabolites. We could also demonstrate that blank EDTA vacutainer tubes contain a significant amount of sarcosine. Finally, we compared the metabolome of young people against elderly subjects and found that the highest number of metabolites significantly changing with age was detected in serum.
Conclusion: Serum samples provide higher sensitivity for biomarker discovery studies. Due to the presence of spurious amount of sarcosine in vacutainer EDTA tubes, plasma EDTA is not suitable for studies requiring accurate quantification of sarcosine.

1. Introduction

Metabolomics is the system level analysis of metabolism driven by the comprehensive identification and quantification of small-molecules in biological systems.

Metabolomics closely reflects the phenotype, since it integrates individual's genetic background, ageing and lifestyle. Metabolomics analysis can be performed in different sample types, such as tissues [1], cells [2–5] and biofluids [6]. In recent years the simultaneous development of biobanking and high-throughput technologies has enabled a new generation of successful large-scale cohort studies where metabolomics can been applied [7–13].

Biobanks can store and distribute to researchers a large amount of blood products. The blood components, plasma and serum, are the most widely used matrices in clinical studies, although there are alternative options of blood collection such as dried blood spots (DBS) [14] and volumetric absorptive microsampling (VAMS) [6] that offer the opportunity of remote collection, without the presence of trained staff. Both plasma and serum are separated from whole blood via centrifugation. Serum collection tubes contain a separation gel and the blood is separated after coagulation has occurred; on the other hand, plasma collection tubes contain anticoagulants, such as EDTA, heparin or citrate, which prevent blood clotting.

Selecting the correct matrix for biochemical analysis is of paramount importance in order to avoid improper diagnosis [15]. Blood collection tubes are often an under-recognized variable in the pre-analytical phase of clinical studies, and it is thus important to evaluate the influences of these matrices during metabolomics analysis [16–18].

Abbreviations: LC-MS, liquid chromatography mass spectrometry; CHRIS, Cooperative Health Research In South Tyrol; FIA, flow-injection analysis; SOPs, standard operating procedures; UHPLC, ultra-high performance liquid chromatography; HILIC, hydrophilic interaction liquid chromatography; PCA, principal component analysis; PC 1, first principal component; PC 2, second principal component; PC 4, fourth principal component; QC, quality control; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; C18:1, octadecenoylcarnitine; PC aa C40:3, 1,2-diacyl-*sn*-glycero-3-phosphocholine 40:3

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Liquid chromatography mass spectrometry (LC-MS) is one of the most popular platform used for metabolomics studies. In literature several reports have explored the influence of collection tubes in metabolic profiling studies, but almost all of them compared untargeted data and not quantitative data [19–21].

The use of commercially available kits for quantitative targeted metabolomics has become popular since it simplifies inter-laboratory comparison and the exchange of data coming from different studies [22]. Among these kits, the Biocrates AbsoluteIDQ® p180 kit, which can be used on a variety of LC-MS/MS instruments, has already been applied to many studies of human serum and plasma, including several large-scale prospective cohort studies [7–9, 11–13, 23]. A recent study compared the influence of plasma EDTA and serum in targeted metabolomics using a similar commercially available kit (Biocrates AbsoluteIDQ® p150) [24].

The aim of this study was to investigate the influence of collection tubes for quantitative targeted metabolomics studies in samples collected during the Cooperative Health Research In South Tyrol (CHRIS) study in order to determine which of these is more suitable for large scale metabolomics studies of the cohort. CHRIS is a population-based study that addresses genetic and molecular basis of common chronic conditions and their interaction with life style and environment in the general population. Several biospecimens, including serum, plasma EDTA and plasma citrate, were collected from participants and stored at the CHRIS biobank [25].

For that, we selected 80 apparently healthy subjects from the CHRIS cohort. These 80 individuals were not known to suffer from any physical or mental disease and were analyzed using the Absolute IDQTM p180 Kit resulting in the measurement of 189 metabolites in each of three investigated matrices.

2. Materials and methods

2.1. Study design and sample collection

The CHRIS study has been described in detail previously [25]. Briefly, the study was carried on in Venosta valley, South Tyrol, Italy. Recruitment was gradual and all 28,000 adults resident in the selected study area were invited to participate, in groups of up to 10 participants per working day.

We included in this study 80 participants enrolled from the first 4979 participants who joined the study between August 24th, 2011 and July 15th, 2014.

A total of 40 independent individuals were selected with age lower than 35 years and assigned to the “young” group. The other 40 independent subjects were selected with age higher than 60 years and assigned to the “elderly” group. All selected individuals were unrelated and free from any diagnosed cardiovascular, metabolic or other chronic disease and did not take any medication on a regular basis. Both groups were sex-matched. In addition, to exclude any potential influence of seasonality, the selection of individuals required participation dates from the same season [26].

Blood was collected at the recruitment center after overnight fasting, and taken in the early morning within a short time interval. We followed WHO guidelines for use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma, and serum samples (WHO/DIL/LAB/99.1 Rev.2 on http://apps.who.int/iris/handle/10665/65957). To ensure measurements' reliability and accuracy, trained nurses performed pre-analytical processing immediately after the drawing. Serum, EDTA and Citrate Vacuette® vacutainer collection tubes were provided by Greiner Bio-One (Kremsmünster, Austria). All vacutainers were inverted five times immediately after blood drawing to mix the anti coagulant or the clot activator with the whole blood.

Serum tubes were allowed to clot for 30 min at room temperature and centrifuged for 15 min at 1500 $\times g$ at RT. Blood tubes with EDTA and citrate were further mixed after blood drawing on a sample rotator for 10 min at room temperature. EDTA tubes had no further treatment, whereas citrate vacutainers were centrifuged for 15 min at 1500 $\times g$ at RT.

EDTA, citrate, and serum tubes from the same day were stored altogether in a transportation bag at room temperature and shipped to the laboratory/biobank. The temperature range during transportation was fixed and monitored through the entire recruitment period. Aliquots of 120 µL were then obtained for serum, plasma EDTA and plasma citrate samples and stored at −80 °C at the Biobank of Bozen/Bolzano until the analysis.

All participants gave written informed consent in accordance with the Declaration of Helsinki. The study received ethical approval by the Ethical Committee of the Healthcare System of the Autonomous Province of Bolzano.

2.2. Chemicals

Acetonitrile was purchased from VWR International (Radnor, PE, USA). Methanol was obtained from Sigma–Aldrich (Seelze, Germany). Water was obtained from a Milli-Q water purification system equipped with LC-pak® polisher (Millipore, USA). All solvents were of LCMS analytical grade or higher purity. NIST plasma EDTA QC sample, Standard Reference Material® 1950 was purchased from NIST (Gaithersburg, MD, USA).

2.3. Liquid chromatography mass spectrometry

Targeted analysis was performed using the Biocrates AbsoluteIDQ® p180 kit with an ultra-high-performance liquid chromatography (UHPLC) (Agilent 1290, Agilent Technologies, Santa Clara, CA, USA) coupled to a Q-Trap mass spectrometer (MS) (QTRAP 6500, Sciex, Foster City, CA, USA).

Sample preparation and analysis were performed according to the manufacturer's protocol. In brief, the sample processing procedure was performed on a single sample and utilized a 96-well plate design where both sample derivatization and analyte extraction were performed. The kit requires 10 µL of sample and provides human plasma based quality controls in 3 concentration levels (low, medium, high) which can be used for quality control purposes and batch normalization. Once processed, each sample undergoes to two separate MS-based analytical runs. A total of 189 metabolites are included in the kit, 43 metabolites are measured by UHPLC-MS/MS and 146 metabolites by flow-injection analysis (FIA)-MS/MS. Isotope-labelled and chemically homologous internal standards are used for quantification providing a total of 56 analytes fully validated as absolutely quantitative. The UHPLC-MS/MS run provide fully validated absolute quantification of 43 metabolites, including 21 amino acids and 22 biogenic amines, with the use of external calibration standards in seven different concentrations and isotope labelled internal standards for most analytes. The remaining 146 metabolites, including 40 acylcarnitines, 90 glycerophospholipids, 15 sphingolipids and 1 sum of hexoses, were analyzed by FIA-MS/MS, using a one point internal standard calibration with representative internal standards (9 isotope-labelled acylcarnitines, 1 isotope-labelled hexose, 1 non-labelled lyso-PC, 2 non-labelled PCs, 1 non-labelled SM, a total of 14 internal standards). 12 acylcarnitines and the sum of hexoses are fully validated as quantitative while for the remaining metabolites analyzed by FIA-MS/MS the kit provides a “semi-quantitative” measurement due to the lack of commercially available specific internal standards. Several lipids analyzed in the present kit represent the total concentrations of possible isobars and structural isomers.”

Sarcosine confirmation was achieved by running the content of blank serum, plasma citrate and plasma EDTA vacutainer tubes with our untargeted MS-based workflows [6]. In brief, 500 µL of water:-methanol (1.1) were added in each vacutainer tube, vortexed 4000 RPM for 5 min, centrifuged at 4300 RPM for 10 Minutes at 20 °C and

transferred in a new tube. Samples were evaporated to dryness under vacuum at 35 °C for 120 min in a vacuum evaporator (EZ − 2, Genevac, Ipswich, UK) and reconstituted with 200 µL of an acetonitrile:water (50:50, v:v) solution.

Samples for sarcosine confirmation were analyzed by ultra-high performance liquid chromatography (UHPLC) (Agilent 1290, Agilent Technologies) coupled to a Q-TOF mass spectrometer (MS) (Triple TOF 5600+, Sciex, Foster City, CA). The chromatographic separation was based on hydrophilic interaction liquid chromatography (HILIC) and performed using an Acquity BEH amide, 100 × 2.1 mm column (Waters Corporation, Milford, MA, USA).

Separation was achieved using acetonitrile +0.1% formic acid as mobile phase A, and water +0.1% formic acid, as mobile phase B. The injection volume was 5 µL and the flow rate was 0.6 mL/min. The following linear gradient was used: 0 min 95% A, 1 min 95% A; 4 min 30% A; 5 min 30% A; 5.1 min 95% A; 8 min 95% A.

The mass spectrometer was operated in full scan mode in the mass range from 50 to1000 *m/z* and with an accumulation time of 250 ms. In ESI+ mode the source temperature was set at 700 °C, the declustering potential at 30 V, the collision energy at 6 V, the ion spray voltage at 5120 V, the curtain gas at 25 psi, the ion source gas 1 and 2 at 60 psi. In ESI- mode the source temperature was set at 650 °C, the declustering potential at −45 V, the collision energy at −6 V, the ion spray voltage at −3800 V, the curtain gas 25 psi, the ion source gas 1 and 2 at 30 psi. The instrument was mass calibrated by automatic calibration infusing the Sciex Positive Calibration Solution (part no. 4460131, Sciex, Foster City, CA) for positive mode and Sciex Negative Calibration Solution (part no. 4460134, Sciex, Forster City, Ca) for negative mode after every two-sample injections.

2.4. Statistical analysis

Metabolites with missing values higher than 80% in all batches were excluded before normalization. Between-batch normalization was performed only on FIA-MS data (acylcarnitines, glycerophospholipids, shingolipids and hexose) using the following equation [22]:

$$\text{Normalized Xij} = \text{Xij} * \frac{\text{mean value (metabolite i) in reference QC in reference batch}}{\text{mean value (metabolite i) in reference QC}}$$

As reference batch, we selected the batch containing serum samples since it had the lowest number of missing values. The manufacturer's quality control (QC) 'medium' - QC2 consisting of a mixture of human plasma spiked with 59 metabolites was used as reference QC.

Metabolites with a coefficient of variation (CV higher than 30%) in QC samples after normalization were excluded. The final dataset in this study was composed of 138 metabolites.

The tube-wise differences were assessed following classical statistical approaches. After replacing missing concentrations with the corresponding limit of detection (in semiquantitative metabolites) or quantification (in quantitative metabolites), a principal component analysis (PCA) was performed to capture both, changes between tubes and a structured variance within samples, in a hypothesis free setting. Subsequently, we investigated tube-dependent abundance changes using an analysis of variance (one-way ANOVA) for each single metabolite.

The PCA analysis was performed on log-transformed concentrations. Therefore, assuming centralized concentrations, we decomposed them into latent structures (PCs), by a singular value decomposition method. We explored the PCs that explained around the 70% of the total variation of the original metabolites.

In the one-way ANOVA, we tested a significant difference among the tubes using the logarithm transformation of the metabolite ensuring the normality assumption that was checked using graphical methods. We investigated the homogeneity of variances graphically and by the Barrlett test. We applied a conservative Bonferroni correction accounting for 138 tested metabolites (p-value = 3.6×10^{-4}).

Furthermore, the biological variability that naturally occurs in the concentration of each metabolite was explored with the CV, to determine if the method used to quantify the metabolite is stable or it varies according to the tube from where it was measured.

In order to describe the data, the pairwise correlations between tubes were explored. After excluding subjects with missing values in at least one tube, the Pearson's correlation coefficient (r) was calculated for each metabolite to compare serum vs plasma EDTA, serum vs plasma citrate, and plasma EDTA vs plasma citrate. Metabolites showing at least one $r < 0.4$ were excluded from further data analysis. The *t*-test on the log-transformed metabolite abundances was performed to compare concentrations in elderly versus young, at significance level of 3.6×10^{-4}.

All statistical analyses were performed with *the R software package version 3.4.2 (www.r-project.org/) using the Rstudio* version 1.0.143 interface (http://www.rstudio.com/). The PCA was fitted using the *prcomp* function. Graphs were plotted using *R* and *MetaboAnalyst* [27].

3. Results

The aim of this study was to evaluate the influence of collection tubes for human blood samples applied to quantitative targeted analysis of metabolic profiles. The final goal was to identify which collection tube is more suitable for large scale metabolomics studies.

Therefore, we designed an experiment that aimed to compare human blood samples from the CHRIS cohort [25] obtained in three different collection tubes, resulting in three separate samples from the same participant, serum, plasma EDTA and plasma citrate, respectively.

We selected 80 apparently healthy subjects from the CHRIS cohort, 40 individuals below 35 years of age and 40 above 60.

We analyzed all samples using the same protocol for targeted metabolomics (Absolute IDQTM p180 Kit) resulting in the measurement of 189 metabolites via LC or FIA coupled to tandem mass spectrometry (MS/MS).

3.1. Preprocessing and normalization

Samples were analyzed in three analytical batches. Each batch was designed to contain 80 samples, 7 calibrators, three plasma quality control (QC) samples provided by the kit supplier, one internal serum QC sample representative of the CHRIS cohort and one commercial plasma NIST QC sample. QC samples supplied as part of the kit by the manufacturer consist of mixtures of human plasma spiked with 59 metabolites (42 and 17 measured by LC and FIA respectively) in 3 nominal concentrations ('low' QC1, 'medium' – QC2, 'high' – QC3). The second QC sample was a reference plasma sample routinely used as QC (NIST SRM 1950) [28]. The third QCs consist of an internal pool of serum samples from the CHRIS cohort [25]. Samples coming from the same collection tube were analyzed within the same analytical batch in randomized order.

In all three collection tubes we detected a similar number of missing values. In plasma citrate we detected 30.8% of missing values, 30.9% in plasma EDTA and 30.1% in serum.

Next, we considered the reproducibility of the quality control (QC) samples to distinguish potential biases and batch effect that could affect our findings. The CV% of all QCs across the three batches showed that only 37% of targeted metabolites showed a CV% lower than 10 (Fig. S1). Of note, the behavior of QCs was quite different in samples analyzed by LC-MS and FIA-MS. Amino acids and biogenic amines in QC samples (analyzed by LC-MS) showed a CV% lower than 10 for 97% and 77% of the analyzed metabolites respectively (Fig. S2), while metabolites analyzed in FIA showed much higher variability, especially for phospholipids and sphingolipids as reported in Fig. S2. These results are in agreement with a previous report by Siskos et al. [22], where it was

found that inter-laboratory comparison of targeted metabolomics data acquired with similar Absolute IDQ® p150 Kit required normalization for samples analyzed in FIA-MS but not for samples analyzed in LC-MS.

Therefore, we applied the same normalization strategy as reported previously [22] and described in material and methods using serum as reference batch and QC samples supplied by the manufacturer (medium– QC2) as reference QCs, as the lowest number of missing values was recorded there.

After normalization, the CV% of amino acids and biogenic amines (LC-MS) was not improved (Fig. S1 and Fig. S2), in contrast, a big improvement was achieved for metabolites acquired in FIA-MS (glycerophospholipids, sphingolipids and acylcarnitines). These results confirmed that normalization is necessary only for data acquired in FIA-MS [22] and was, therefore, applied only on data acquired in FIA-MS.

3.2. Comparison of serum, plasma EDTA and plasma citrate

We next evaluated the influence of collection tubes for targeted metabolomics. We first applied a principal component analysis (PCA) on the dataset. The first two components (PC 1 and PC 2) accounted for 48.9% and 9.3% respectively, of the total variance of the dataset and did not show any effect due to sampling performed using different collection tubes (Fig. 1a). However, exploring the other principal components revealed that the fourth component (PC 4) accounted for 5.5% of the total variance in the dataset, and separated the samples collected in serum from the samples collected in plasma citrate and plasma EDTA (Fig. 1b). Therefore, even if minimal, collection tubes account for some variance in the dataset, suggesting that the metabolome in serum differs from the one obtained from both plasma citrate and plasma EDTA.

Moreover, samples collected in plasma citrate seemed to cluster more tightly suggesting a lower biological variation (Fig. 1). To confirm this, we compared the inter-subject variability obtained in each collection tube for the 80 subjects included in this study. We plotted the logarithmic value of the geometric mean against the CV for each metabolite and found that EDTA and serum samples have very similar biological variation but not citrate (Fig. 2). Overall, the inter-subject variability in citrate was lower considering that 75% of metabolites had a CV lower than 30% in the 80 subjects investigated. On the other hand, 40% of all metabolites in EDTA samples and 36% in serum had CV lower than 30% (Fig. S3). While the biological variation was similar for the amino acids in the three collection tubes, the inter-subject variability for lipids (glycerophospholipids and- sphingolipids) was much smaller in citrate (Fig. 2). For glycerophospholipids, the inter-subject variability in citrate resulted in 68% of metabolites with CV lower than 30%, while 25% of all metabolites in EDTA samples and 19% of metabolites in serum had CV lower than 30%. For sphingolipids, 93% of metabolites had a CV lower than 30%, while 7% of all metabolites in EDTA samples and 20% of metabolites in serum had CV lower than 30% (Fig. 2).

These biological differences, however, do not explain the cluster observed in the fourth component during PCA (Fig. 1b). To better understand the causes of this clustering of serum samples and which metabolites were contributing to it, we therefore performed a correlation analysis and calculated the relative concentration differences for each individual metabolite for the three different collection tubes.

As a general trend, we observed that metabolite levels were higher in serum than in plasma citrate and EDTA (Fig. 3). In particular, for amino acids and biogenic amines, we recorded higher concentrations in serum. For amino acids, the average value of the relative difference was 7% higher in serum than EDTA and 6% higher in serum than citrate. For biogenic amines the average value of the relative difference was 11% higher in serum than EDTA and 15% higher in serum than citrate. Moreover, the average Pearson's correlation coefficients (R) were always higher than 0.7 for biogenic amines and amino acids (Table S1).

More specifically, we found that 17 metabolites were significantly changing among different collection tubes (Anova test with Bonferroni correction), 9 of them being amino acids, 6 biogenic amines, 1 glycerophospholipid and 1 acylcarnitine (Table 1, Fig. 3, Fig. 4, Fig. S4, S5 and S6). Aspartic acid and serotonin were only detected in serum and the highest relative differences were obtained for arginine and taurine. Arginine was found to be 27% higher in serum than EDTA and 26% higher in serum than citrate, while taurine resulted to be 39% higher in serum than EDTA and 38% higher in serum than citrate (Table 1 and Fig. 3 and Fig. 4). Glutamine, serine, histidine, lysine, phenylalanine, methionine and kynurenine also showed greater values in serum than plasma citrate and EDTA (Table 1, Fig. 4, and Fig. S4, S5 and S6). The level of ADMA, SDMA and glutamic acid were comparable in serum and

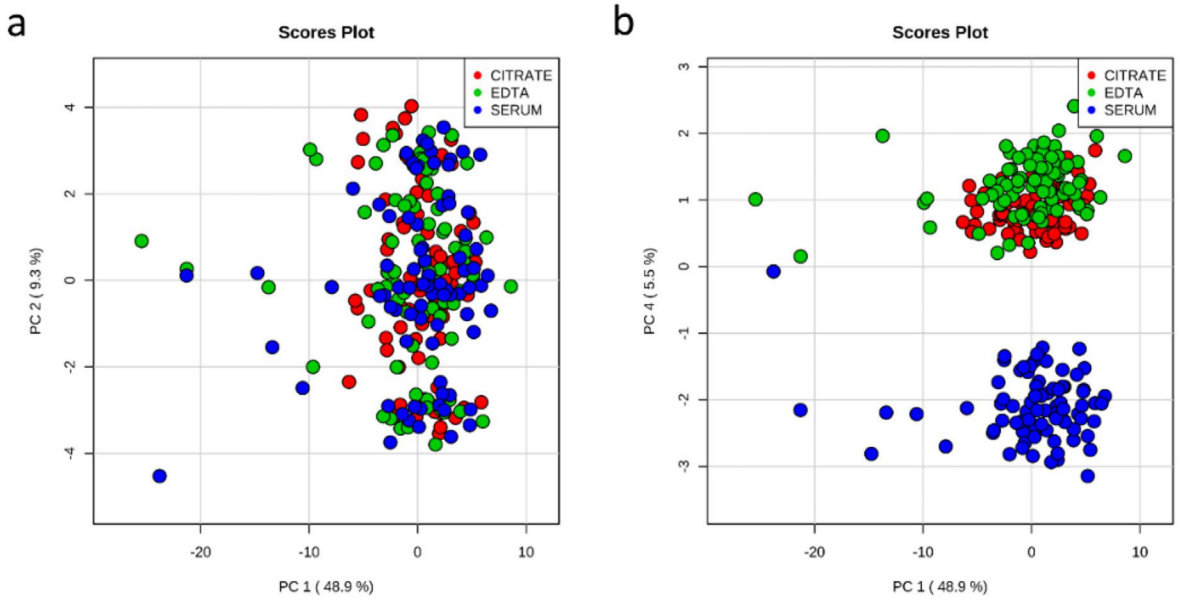

Fig. 1. Principal component Analysis (PCA). PCA was performed on targeted metabolomics data of serum, plasma EDTA and plasma citrate collected from the same 80 participants. a) Plot of the second principal component (PC 2) versus the first principal component (PC 2). b) Plot of the forth principal component (PC 4) versus the first principal component (PC 4).

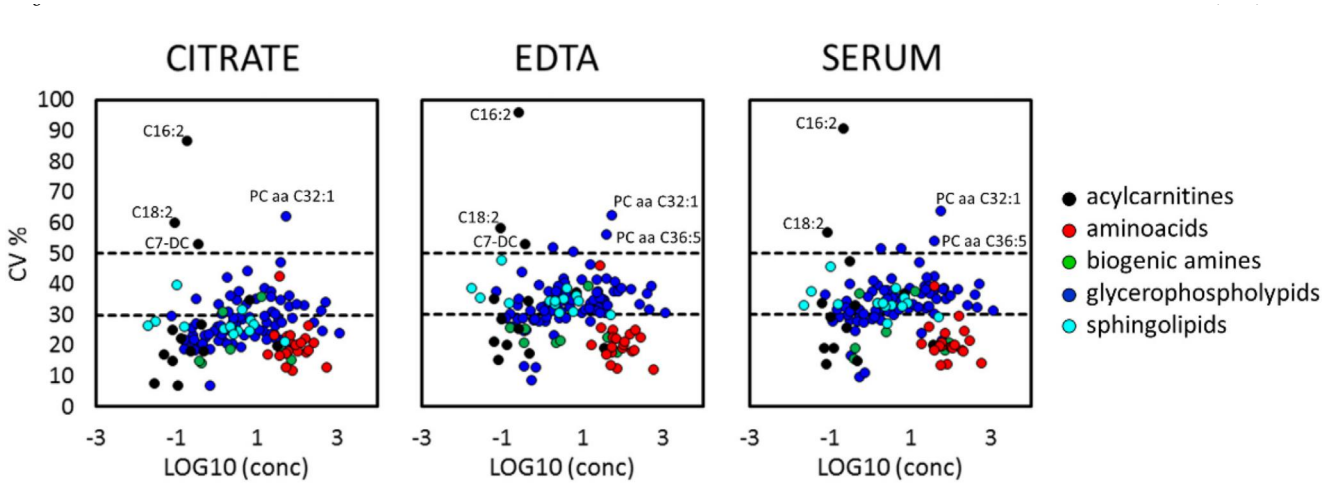

Fig. 2. Biological variation. Difference in inter-subject variability measured by the coefficient of variation in percentage (CV %) as function of the concentration (log10(geometric mean)) for each metabolites analyzed in the three different collection tubes.

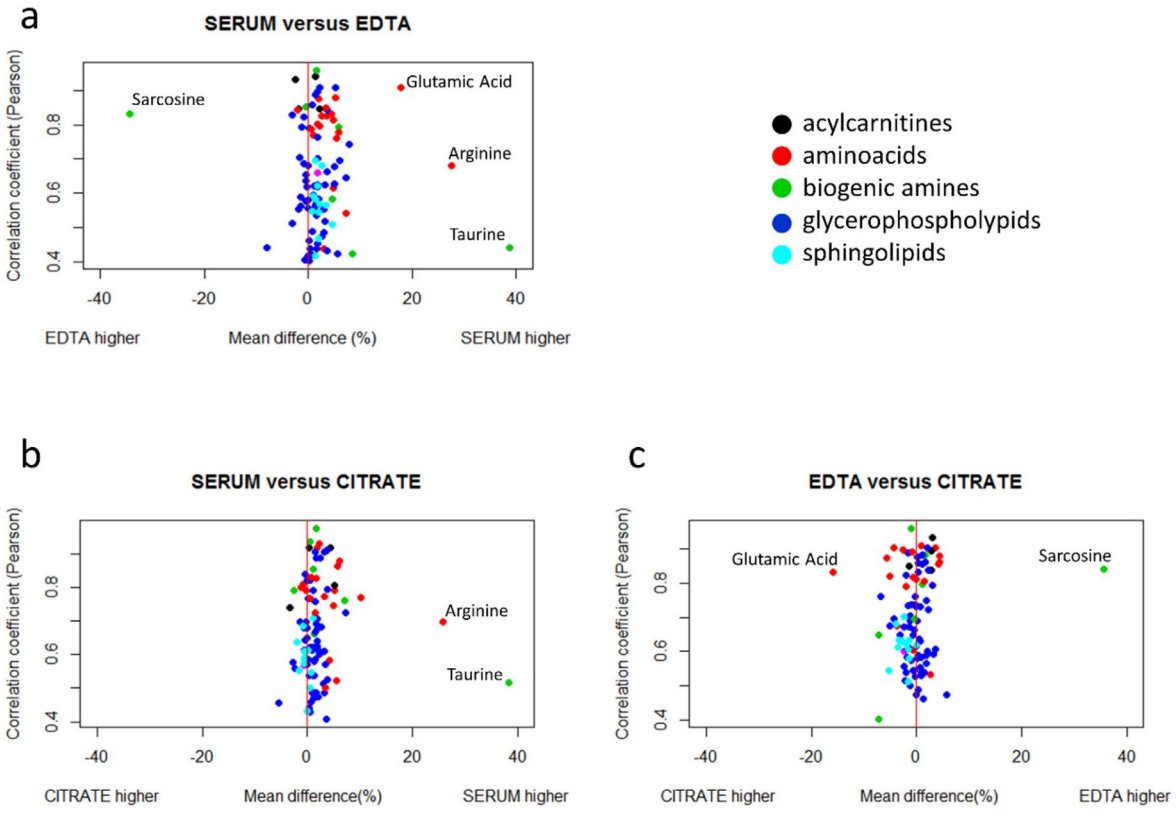

Fig. 3. Relative concentration difference and correlation coefficients. The x-axis indicates the mean value of the relative concentration difference and the y-axis indicates the correlation coefficients for each metabolites. a) Relative concentration differences and correlation coefficients were calculated comparing serum versus EDTA samples. b) Relative concentration differences and correlation coefficients were calculated comparing serum versus citrate samples. c) Relative concentration differences and correlation coefficients were calculated comparing EDTA versus citrate samples.

plasma citrate but were lower in EDTA, while the level of the acylcarnitine C6 (C4.1-DC) were higher in citrate than serum and plasma EDTA (Table 1, Fig. 4, and Fig. S4, S5 and S6).

The only two metabolites that were found at greater concentration in EDTA were sarcosine and PC ae (42:3). In particular, sarcosine was shown to be 34% higher in EDTA than serum and 36% higher in EDTA than citrate (Table 1, Fig. 3, and Fig. 4). We used high resolution mass spectrometry and analyzed the content of blank vacutainer tubes used to collect serum, plasma EDTA and plasma citrate samples. Surprisingly, we found that blank EDTA tubes contain a significant amount of sarcosine, and confirmed the identity of this molecule using accurate mass and tandem mass spectrometry (Fig. 5). No sarcosine was detected in blank serum and plasma citrate tubes (Fig. 5). None of the other metabolites that were found changing significantly among matrices (Table 1) were found in the blank tubes tested.

Finally, to investigate the impact of different collection tubes when analyzing big cohorts, we compared the metabolome of apparently healthy young people against the metabolome of apparently healthy elderly subjects. Using univariate statistics (*t*-test with Bonferroni correction) we found that the highest number of metabolites significantly

Table 1
Metabolites changing among different collection tubes (ANOVA test with Bonferroni correction).

Classes	Metabolites	ANOVA	SERUM-EDTA		SERUM-CITRATE		EDTA-CITRATE	
		p-Value	r	Mean diff %	r	Mean diff %	r	Mean diff %
Aminoacids	Arginine	1.1E−42	0.7	27	0.7	26	0.8	−2
Aminoacids	Lysine	4.3E−04	0.8	5	0.8	5	0.7	0
Aminoacids	Histidine	1.0E−05	0.6	5	0.6	4	0.6	−1
Aminoacids	Phenylalanine	6.6E−10	0.5	7	0.5	3	0.7	−4
Aminoacids	Glutamine	1.4E−06	0.4	3	0.5	6	0.5	3
Aminoacids	Methionine	1.2E−06	0.4	8	0.4	3	0.4	−5
Aminoacids	Serine	9.5E−09	0.8	6	0.8	10	0.9	4
Aminoacids	Glutamate	2.7E−09	0.9	18	0.9	2	0.8	−16
Aminoacids	Aspartate	2.5E−103	NA		NA		NA	
Biogenic amines	Kynurenine	1.7E−04	0.8	6	0.8	7	0.8	1
Biogenic amines	Taurine	5.2E−79	0.4	39	0.5	38	0.7	0
Biogenic amines	SDMA	1.8E−05	0.6	5	0.8	−3	0.7	−7
Biogenic amines	Serotonin	7.6E−88	NA		NA		NA	
Biogenic amines	ADMA	2.1E−09	0.4	9	0.7	1	0.4	−7
Biogenic amines	Sarcosine	1.5E−56	0.8	−34	1.0	2	0.8	36
Acylcarnitines	C6 (C4:1-DC)	2.1E−07	NA		NA		NA	
Glycerophospholipids	PC ae (42:3)	3.8E−06	0.4	−9	0.4	1	0.5	10

Abbreviation: r, Pearson's correlation coefficient.

changing with age was detected in serum. Indeed, 6 metabolites were observed to be higher in elderly subjects in serum, namely citrulline, ornithine, ADMA, SDMA, kynurenine and C18:1 (Fig. 6). The same analysis resulted in only three metabolites changing in EDTA, citrulline, ornithine, kynurenine (Fig. 6). In plasma citrate the metabolites changing were five. Citrulline, SDMA, C18:1, PC aa C40:3 were increased in elderly subjects while methionine was higher in young participants (Fig. 6).

4. Discussion

Standardization is a pivotal aspect in any omics technology. Harmonization of procedures to ensure pre-analytical and analytical robustness is mandatory in clinical metabolomics [29]. The use of commercially available kits for quantitative targeted metabolomics can help the harmonization of clinical metabolomics since it simplifies inter-laboratory comparison and the exchange of data coming from different studies [21]. Nevertheless, considering that most clinical metabolomics studies include data from different blood samples, plasma EDTA, plasma citrate and serum, the selection of the appropriate matrix is of great importance to ensure proper clinical translation.

For this reason, this study investigated the influence of different collection tubes on the composition of the metabolome of human blood samples available in the CHRIS biobank [25]. In this study, we did not evaluate plasma heparin collection tubes as matrix for targeted metabolomics since this blood sample was not included in the CHRIS cohort. Plasma heparin collection tubes has been recently evaluated for untargeted metabolomics [21] and lipidomics [19] proving that this collection tube might be suitable for metabolomics application.

In general, we observed that serum samples contain higher levels of several metabolites compared to plasma citrate and EDTA. This was particularly true for several aminoacids and biogenic amines. Higher concentrations in serum compared to EDTA were previously reported

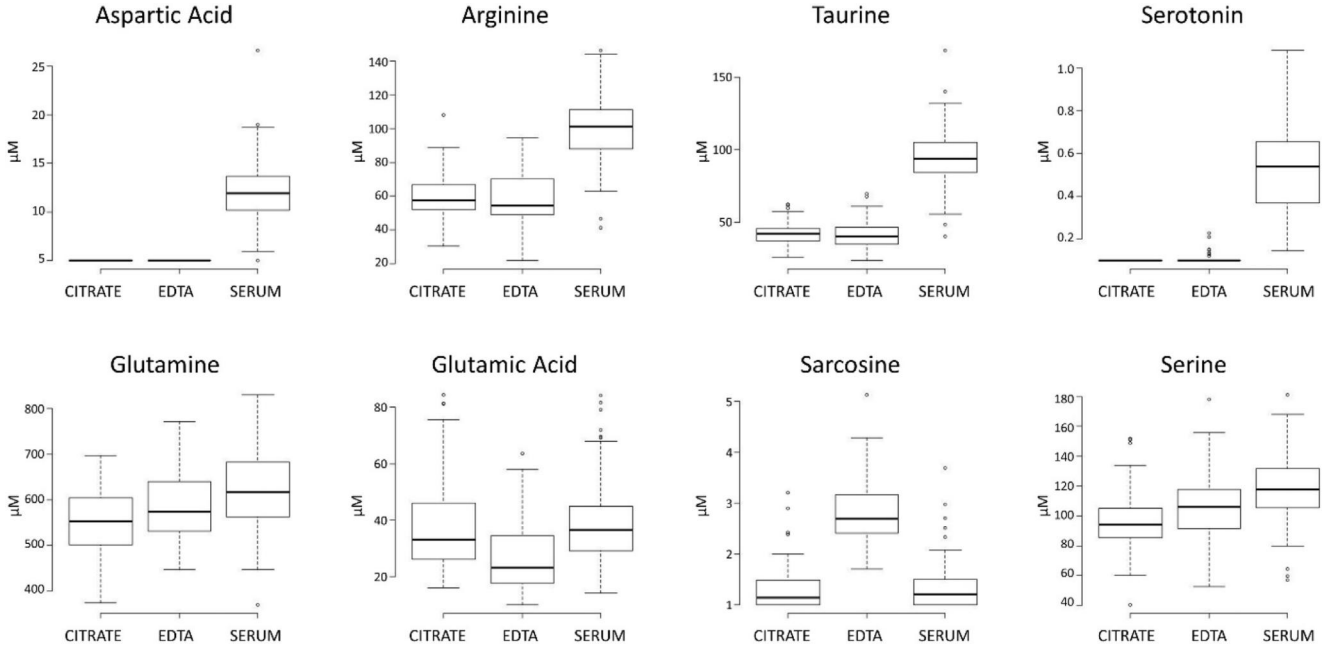

Fig. 4. Metabolites levels different collection tubes. Boxplot diagrams of selected metabolites changing among serum, EDTA and citrate.

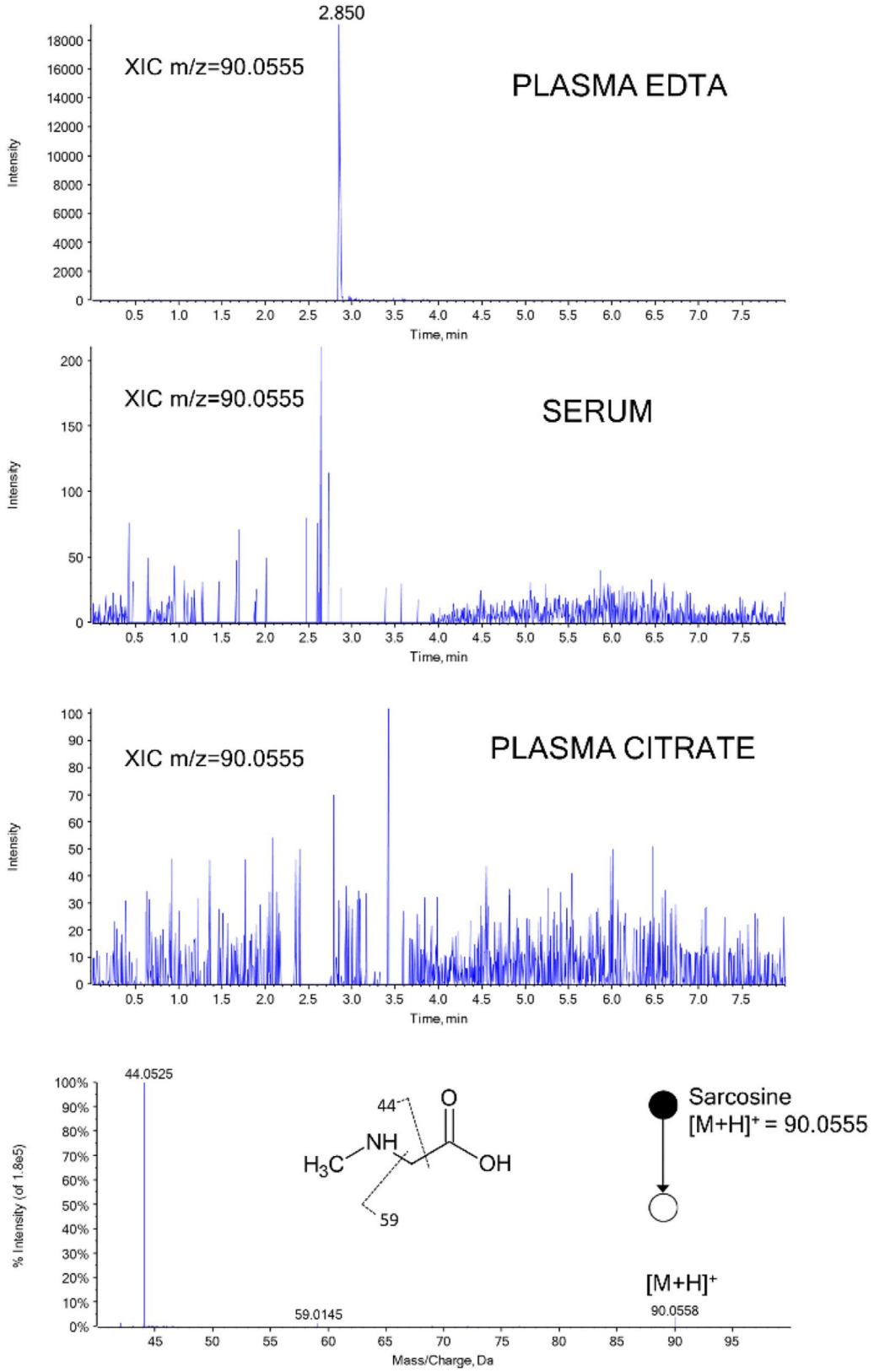

Fig. 5. Presence of significant amounts of sarcosine in EDTA collection tubes. Sarcosine was detected and confirmed by tandem mass spectrometry and accurate mass in plasma EDTA collection tubes.

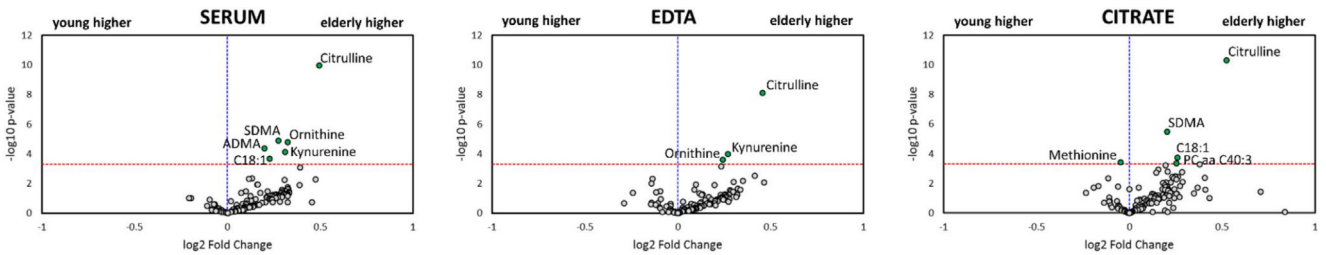

Fig. 6. Volcano plots of ageing. Volcano plots obtained by comparing 40 apparently healthy young people vs 40 apparently healthy elderly people in different collection tubes.

for some of these metabolites, such as arginine [24, 30] and ADMA and SDMA [30], but these studies did not include a comparison with plasma citrate. In addition, our analysis identified other metabolites with significant higher concentrations in serum compared to plasma EDTA and citrate, such as aspartic acid, kynurenine, taurine and serotonin.

Higher levels of metabolites in serum samples might be explained by the combination of two factors. First, in serum samples a fraction of proteins was removed during sample collection and pre-analytic procedures resulting in a smaller total volume and thus the metabolites were more concentrated. Second, during coagulation of serum samples, some metabolites were likely released from blood cells, in particular from activated platelets, increasing their concentration [30, 31].

Nevertheless, higher metabolite levels might also be due to biological variation. Indeed, the correlation analysis among matrices provided high Pearson's correlation coefficients (R) for biogenic amines and amino acids indicating that differences of metabolite levels between collection tubes were mainly due to systemic changes across all individuals.

Once again, serum samples showed higher biological variation, which might be partially explained considering the issues described above (more complicated collection procedure that requires time for coagulation and might release variable amounts of some metabolites during clotting). Nevertheless, a similar biological variation was found in plasma EDTA but not in plasma citrate. Therefore, our results suggest that a combination of background noise, which differs between collection tubes, and pre-analytical procedures might affect the accuracy of the measurement, especially for metabolites that are present in blood at low concentrations. Of the three matrices, plasma citrate samples provided the lowest biological variation, especially lipids, suggesting that this matrix might be a better choice for lipidomics.

Of interest, the behavior of sarcosine was different compared to all other metabolites analyzed in this study. Indeed, sarcosine was found to be significantly higher in plasma EDTA compared to serum and plasma citrate, suggesting the presence of spurious amounts of sarcosine in the EDTA tubes used for sample collection. We verified this hypothesis, by analyzing the content of blank vacutainer tubes used to collect serum, plasma EDTA and plasma citrate samples confirming that sarcosine is present only in blank vacutainer plasma EDTA tubes. This is especially alarming since sarcosine has recently been identified as a potential biomarker for invasive prostate cancer [32]. Our findings suggest to avoid plasma EDTA samples for accurate quantification of sarcosine in future studies due to the presence of spurious amounts of sarcosine in the vacutainer EDTA tubes.

Given the higher level of metabolite concentrations in serum (and higher biological variation), this matrix might provide higher sensitivity for biomarker discovery studies in large metabolomics studies. As proof of principle, we compared the metabolome of apparently healthy young people against the metabolome of apparently healthy elderly subjects. Our result confirmed that serum contains the highest number of metabolites significantly changing with age.

Notably, some of these metabolites, such as citrulline were recently reported as biomarkers for ageing [33, 34], but due to a limited sample size, these results need to be replicated in bigger cohorts.

Taken together, these results demonstrate that, possibly because of higher concentrations detected, serum is a better matrix for biomarker discovery studies.

5. Conclusion

In this study we evaluated the influence of collection tubes for human blood samples during quantitative targeted metabolomics. As a general trend, we observed that metabolite concentrations were higher in serum than plasma citrate and EDTA, but, considering that most of the metabolites showed a high correlation between tubes, the selection of serum, plasma EDTA or plasma citrate for quantitative metabolomics analysis does not necessarily introduce a significant bias during epidemiological studies. Serum however, due to higher concentrations detected, provides higher sensitivity for biomarker discovery studies. Interestingly, plasma citrate samples provide the lowest biological variation for lipids in the CHRIS collection, suggesting this matrix be better suited for quantitative targeted metabolomics of lipids. And lastly, plasma EDTA samples contained the lowest concentrations and showed a biological variation similar to serum samples. Moreover, it was found that Vacutainer EDTA tubes contain spurious amount of sarcosine, suggesting that this matrix is not suitable for studies requiring accurate quantification of sarcosine.

Finally, citrulline, SDMA, C18:1, PC aa C40:3 were increased in apparently healthy elderly subjects compared to apparently healthy young participants, encouraging further ageing biomarkers discovery in larger cohorts.

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Data statement

The dataset is part of the CHRIS study [25] and is available on request.

Supplementary data

Supplementary material

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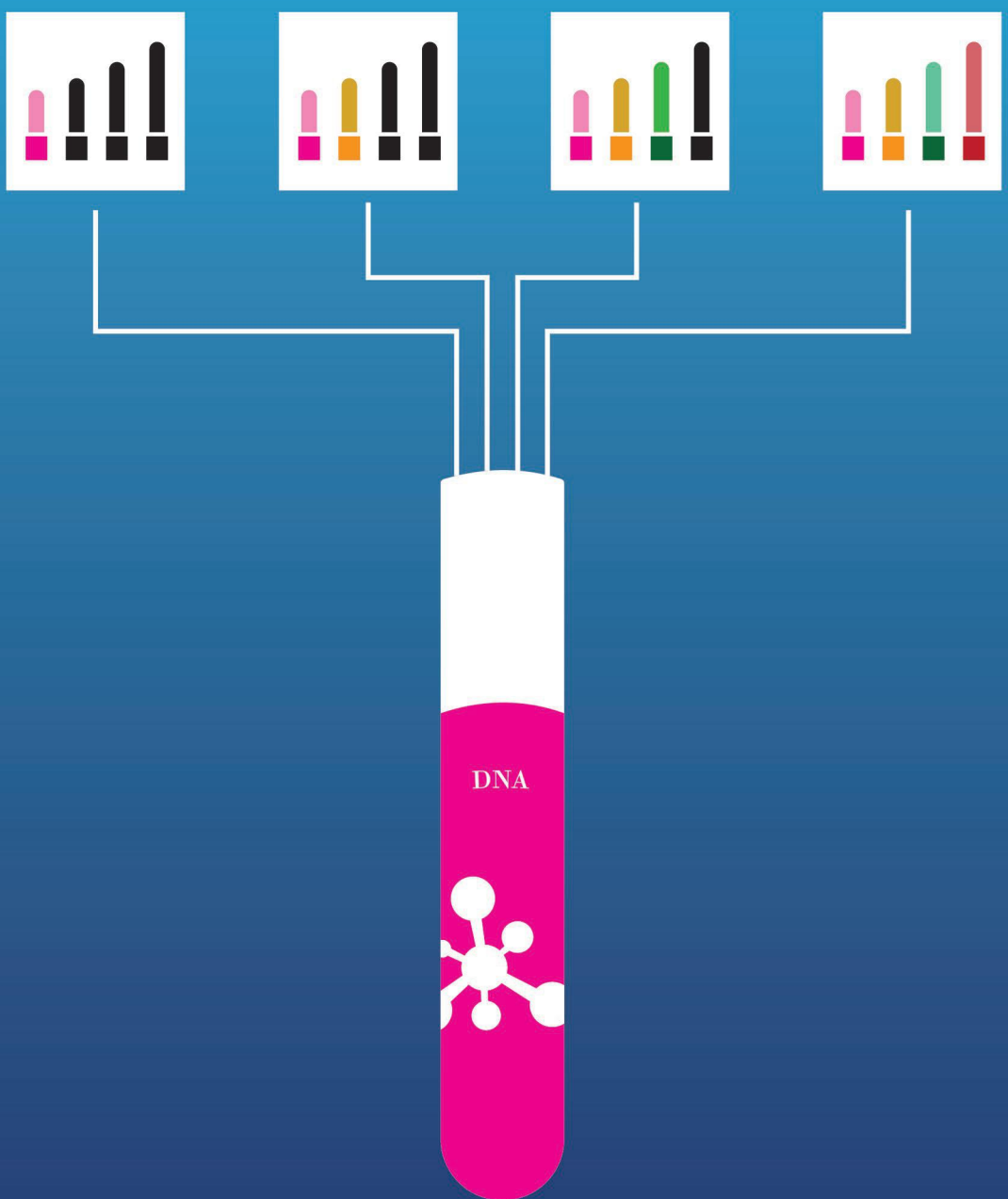

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