·临床研究·

白细胞过滤器对红细悬液中血小板的去除效率

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摘　要：【目的】观察不同储存时期红细胞悬液中血小板数量的变化，探讨白细胞过滤器对红细胞悬液中的血小板去除效率。【方法】将 58 份红细胞悬液根据储存时间不同分为 4 组：1 周组(16 份)，2 周组(16 份)，3 周组(14 份)，4 周组(12 份)，将四组红细胞悬液通过白细胞过滤器过滤，获取过滤前后的样本，用全自动血细胞计数仪检测血小板计数，计算出血小板的去除率。过滤前红细胞悬液标本制成血细胞涂片，用吉姆萨染色液染色，显微镜下观察血小板的形态。【结果】四组红细胞悬液中过滤前血小板的计数分别为(286.5±62.34)×10^9/L、(238.0±57.37)×10^9/L、(193.6±56.21)×10^9/L 和(167.8±24.76)×10^9/L，储存 3 周(P <0.01)和 4 周(P <0.0001)组血液中的血小板计数明显低于储存 1 周组。不同储存时期红细胞悬液血细胞涂片中，均可以观察到成群分布和散在分布的形态正常的血小板。4 组红细胞悬液的血小板去除率分别为(80.13±9.06)%，(76.41±10.13)%，(77.78±9.30)% 和(70.63±9.39)%，各组之间血小板去除率比较差异无统计学意义(P >0.05)。【结论】红细胞悬液中的血小板计数随着储存时间的延长而逐渐降低，而储存后期(3 周和 4 周)仍保存有大部分的血小板，白细胞过滤器能够去除其中的绝大多数血小板，而且各组间去除效率相似。

关键词：红细胞悬液；白细胞过滤器；储存时间；血小板去除；效率
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Platelet Removal Efficiency by Leukocyte Filter in Red Blood Cell Units at Different Storage Periods

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Abstract:【Objective】The aim of this study is to investigate change of platelet count in red blood cell (RBC) units at different storage periods and explore the efficiency of platelet removal by leukocyte filter.【Methods】A total of 58 RBC units were divided into four groups according to different storage periods: 1 week Group (16), 2 weeks Group (16), 3 weeks Group (14) and 4 weeks Group (12). RBC units in the four groups were filtered through leukocyte filter. The RBC samples before and after filtration were obtained. The platelet count was detected by automatic blood cell counter and the efficiency of platelet removal was calculated. RBC samples before filtration were made into blood cell smears. The blood cell

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smears were dyed with Wright-Giemsa stain, and the morphology of platelets was observed through a microscope.【Results】The platelet count in RBC units stored for 1, 2, 3 and 4 weeks was (286.5±62.34)×10⁹/L, (238.0±57.37)×10⁹/L, (193.6±56.21)×10⁹/L and (167.8±24.76)×10⁹/L, respectively. Platelet count in blood stored for 3 weeks (P<0.01) and 4 weeks (P <0.000 1) were significantly lower than those stored for 1 weeks. When observed in the blood smears of RBC units at different storage periods, platelets with normal morphology were distributed in clump and scattered style. The platelet removal rates of the four groups were (80.13±9.06) %, (76.41±10.13) %, (77.78±9.30) % and (70.63±9.39) %, respectively, with no significant difference (P >0.05).【Conclusions】Platelet count in RBC units decreases gradually as the storage period increases, but most platelets still remain in RBC units of late storage periods (3 weeks and 4 weeks). The leukocyte filter is able to remove most of the platelets, and the removal efficiency is similar among the groups.

Key words: red blood cell; leukocyte filter; storage period; platelet removal; efficiency

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临床应用的红细胞悬液中除了用于提高血红蛋白含量的红细胞外,还有少量的白细胞和血小板。其中的白细胞极少具有治疗价值,但可能引起严重的输血反应[1-3];而其中的血小板的输入可能引起血小板同种免疫[4]。从离心法到细胞过滤法,去除白细胞的方法已经使用了几十年[5]。第三代白细胞过滤器从血液成分中去除大于3 log(99.9%)的白细胞,降低输血相关不良反应的发生率[5]。有研究表明红细胞悬液中白细胞的过滤与血小板有关[6],那么白细胞过滤器在滤除白细胞的同时是否能减少血小板?本研究将检测不同储存时期红细胞悬液中血小板数量的变化以及白细胞过滤器对血小板去除的效率。

1 材料与方法

1.1 研究设计

本研究经中山大学附属第三医院伦理委员会批准(批准文号:2022-208)。红细胞悬液均来自广州血液中心,分为四组:保存1、2、3、4周的红细胞悬液分别归为第1、2、3、4组。红细胞悬液的保存温度为(4±2)℃,红细胞添加剂为CPDA (citrate phosphate dextrose adenine),储存期为35 d。在专用实验室过滤红细胞悬液,过滤前和过滤后计量红细胞的容积,并且获取样本进行相关检测。

1.2 材料

白细胞过滤器(FTS-RC 301或FTS-RC 302,南京双威生物技术有限公司,FTS-RC 301和FTS-RC 302的过滤器孔径相同,转移袋的容量不同)。全自动血细胞计数仪(BC-5000,深圳迈瑞生物医疗电子股份有限公司),高频热合机(GZR-Ⅲ,苏州市医用仪器厂),吉姆萨染色剂(BA-4017,珠海巴索生物科技有限公司),显微镜(CX43,奥林巴斯,日本)。

1.3 红细胞悬液的过滤

1单位红细胞悬液用FTS-RC301过滤,1.5或2单位红细胞悬液用FTS-RC302过滤。无菌消毒后,将FTS-RC 301或FTS-RC 302通过针刺插入红细胞悬液血袋,血液通过重力流过滤到转移血袋中。从每袋红细胞悬液中获取过滤前后的样本进行血小板计数。

血小板去除率的计算算式如下:

血小板去除率=(过滤前血小板计数×过滤前红细胞悬液的容积-过滤后血小板计数×过滤后红细胞悬液的容积)÷(过滤前血小板计数×过滤前红细胞悬液的容积)×100%

1.4 血小板形态检测

将一滴血液放在干净的载玻片上,用另一个载玻片将血液涂成均匀的单层。将载玻片风干,放在染色架上。血液载玻片按照生产厂家说明书进行染色。首先用足够的染色剂淹没载玻片并染色1分钟;然后小心地加入pH值为6.8的缓冲液,不要溢出,轻轻混合,直到形成绿色的金属光泽;然后用蒸馏水冲洗载玻片,风干,用显微镜观察血小板形态。

1.5 统计分析

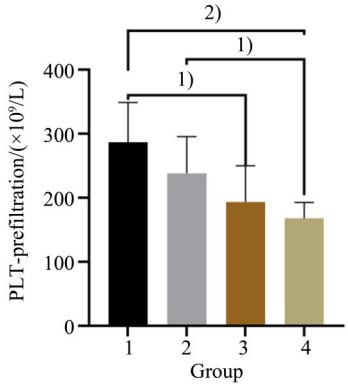
本实验的数据均符合正态分布,各组的方差具有齐性。数据采用SPSS 20.0软件进行单因素

ANOVA统计分析。数值以均值±标准差表示，P<0.05表示差异有统计学意义。

2　结　果

2.1　不同储存时期红细胞悬液中血小板计数的变化

1~4组分别包含16、16、14和12例样本。4组红细胞悬液中血小板的计数分别为286.5±62.34×10^9/L，238.0±57.37×10^9/L，193.6±56.21×10^9/L和167.8±24.76×10^9/L，经方差分析，4组间差异有统计学意义（F=7.102，P=0.000 6），采用Tukey法作两两比较，发现第1组与第3组、第4组间比较差异均有统计学意义（P=0.001 1，P<0.000 1），第2组与第4组比较差异也有统计学意义（P=0.001 4；图1）。



Automatic blood cell counter showed platelet count in RBC units decreased gradually as the storage period increased. 1) P <0.01 for Group 1 compared with Group 3, and for Group 2 compared with Group 4, 2) P <0.000 1 for Group 1 compared with Group 4. n=16, 16, 14 and 12 in Groups 1, 2, 3 and 4.

图1　不同储存时期红细胞悬液中血小板计数的变化

Fig. 1　Changes of platelet count in RBC units during different storage periods

2.2　不同储存时期红细胞悬液中血小板的形态

在显微镜下，储存1~4周的红细胞悬液标本制成的血细胞涂片中均可以观察到血小板，其形态正常，呈成群分布和散在分布两种形式（图2）。

2.3　白细胞过滤器对红细胞悬液中血小板的过滤效率

经白细胞过滤器过滤后，4组红细胞悬液中残留的血小板计数为63.56±31.14×10^9/L，64.00±32.18×10^9/L，49.36±22.75×10^9/L和55.17±18.44×10^9/L，经方差分析，4组间差异无统计学意义（F=0.975 8，P=0.411 0）。各组血小板去除率分别为（80.13±9.06）%，（76.41±10.13）%，（77.78±9.30）%和（70.63±9.39）%，经方差分析，4组间差异无统计学意义（F=2.396，P=0.078 3；图3）。

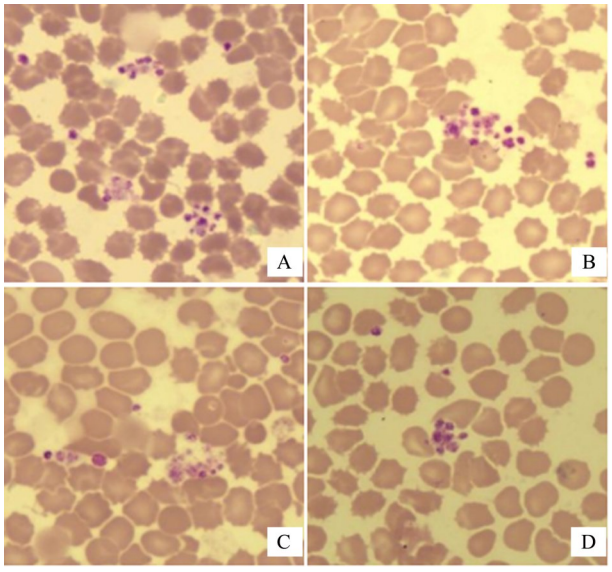


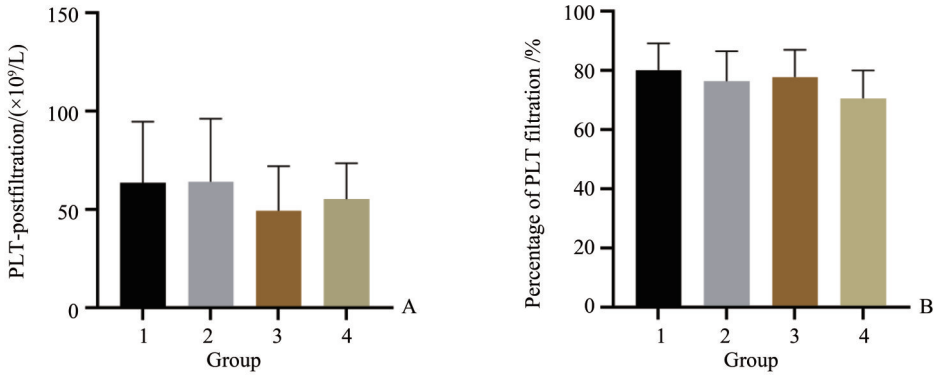
Fig. 2 showed that platelets with normal morphology were observed in the blood smears of RBC units at all different storage periods. RBC samples before filtration were made into blood cell smears. The blood cell smears were dyed with Wright-Giemsa stain and the platelets were observed through microscopy. A: Platelets in RBC units stored for 1 week ×1 000; B: Platelets in RBC units stored for 2 weeks ×1 000; C: Platelets in RBC units stored for 3 weeks ×1 000; D: Platelets in RBC units stored for 4 weeks ×1 000.

图2　不同储存时期红细胞悬液中血小板的形态

Fig. 2　Morphology of platelets in RBC units at different storage periods

3　讨　论

随着血小板在临床的广泛应用，医务工作者发现有时候血小板输注后其增加量低于预期值，这就是血小板无效输注（platelet refractoriness，PTR）[7-8]。PTR的原因可分非免疫因素和免疫因素[9-10]。免疫因素包括怀孕、输血、移植等，这些因素使血小板暴露于人白细胞抗原（human leukocyte antigen，HLA）和/或人血小板特异性抗原（human platelet antigens，HPA）的异体免疫[10]。临床所用的红细胞悬液中存在白细胞和血小板，白细胞表面表达有HLA，血小板表面同时表达HLA及HPA。患者通



RBC units were filtered by leukocyte filter and platelet in the postfiltration units were tested by automatic blood cell counter. A: Platelet count in postfiltration RBC units of different storage periods; B: Efficiency of platelet removal. *n*=16, 16, 14 and 12 in Groups 1, 2, 3 and 4.

图3 白细胞过滤器对不同储存时期红细胞悬液中血小板的过滤效率

Fig. 3 Efficiency of platelet removal in RBC units of different storage periods by leukocyte filter

过输红细胞悬液接受同种异体的HLA和/或HPA后,可能通过同种免疫产生HLA和/或HPA的抗体,当再次输注表达有相应抗原的血小板时,就会出现免疫相关性PTR。有研究表明,在实体肿瘤以及血液病患者中,PTR发生率达7%~34%[11]。

为减少同种免疫,贫血的患者在输注红细胞时,大多选择去白细胞的红细胞,目前主要采用过滤法去除红细胞悬液中的白细胞[12-13]。白细胞过滤器去除白细胞的过程涉及到多种机制,间接黏附是其中的机制之一[6]。血小板在储存过程中逐渐活化而表达黏附分子如P-选择素,而P-选择素可通过其C型凝集素样结构域帮助粒细胞、单核细胞和淋巴细胞亚群黏附至血小板[14]。此外,活化的血小板能释放血小板微粒(platelet microparticles, PMP),PMP同样表达功能黏附分子包括P-选择素,从而与表达有P-选择素糖蛋白配体-1(P-selectin glycoprotein ligand-1, PSGL-1)的白细胞结合。体外实验表明,PMP能够增加流动的HL-60细胞或中性粒细胞对预结合到平行板流动小室表面的HL-60细胞或中性粒细胞的瞬时黏附,从而促进白细胞在流动小室的沉积[15]。由此可见,红细胞悬液流经白细胞过滤器时,活化的血小板黏附和扩散到过滤材料上,随后白细胞被血小板捕获而阻留在过滤器中[6],在此过程中白细胞和血小板一起得到了去除,这就解释了本实验中原设计用于过滤白细胞的过滤器应用于红细胞悬液时,其中的大部分血小板也同时得到去除的原因。

本研究发现虽然随着储存时间的延长,红细胞悬液中血小板数量逐渐降低,但是在第3周和第4周仍分别有(193.6±56.21)×10⁹/L和(167.8±24.76)×10⁹/L的血小板(图1)。红细胞悬液保存(4±2)℃条件下,其中的血小板代谢率低,其储存有效期明显长于目前临床所用的室温保存(22±2)℃的血小板[16]。Hanne等[17]研究发现低温保存的血小板储存21 d后仍具有良好的血小板功能。另有研究报道,全血储存21 d后用血栓弹力图检测其血小板功能,结果显示最大血栓形成幅度(MA值)仍高达新鲜全血的90%[18]。我们的实验也发现,在储存3W和4W的红细胞悬液中,显微镜下可见较多的形态正常的血小板,呈成群和散在分布(图2),它们是输血时同种异体HLA的来源之一以及HPA的来源。HLA抗原具有高度的免疫原性,是导致免疫相关性PTR的主要原因[19-20],而HPA抗体通常与HLA抗体同时被检测到[21]。可见,红细胞悬液中的血小板如果未经去除,输注到患者体内后可能通过同种免疫产生抗体,从而增加PTR的发生率。

本实验中使用的白细胞过滤器能够去除红细胞悬液中70%以上的血小板,对不同储存时期的悬浮红细胞,其血小板去除效率差异无统计学意义。因此,我们建议在临床输血工作中,有发生PTR风险的患者申请输注红细胞悬液时,红细胞悬液均进行白细胞过滤器过滤,这不仅能够达到过滤白细胞的效果,而且能够去除掉大部分血小板,从而可能降低HLA和HPA相关的同种免疫所致的PTR的发生率。

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（编辑　余　菁）