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不同全血过滤方法用于去白细胞血液制备的临床对照研究*

欧斐¹ 巩月英² 张丽¹ 马兰¹ 宋燕¹

(1 青海省血液中心 青海 西宁 810000; 2 青海省人民医院 青海 西宁 810000)

摘要 目的:观察不同全血过滤方法用于去白细胞血液制备的效果。**方法:**采用两种全血过滤方法进行对比研究,对照组采用常规法,将采集后全血混匀后直接与白细胞滤器连接直接过滤;实验组采用湿润滤盘法,血液采集完成混匀后静置,先用上层血清10~20 mL湿润滤盘,再混匀与白细胞滤器连接后进行过滤。比较两组制备方法所用的过滤时间、血液回收率、过滤前后血液指标情况及24小时内溶血的发生情况。**结果:**两组全血过滤方法过滤前后白细胞、红细胞、血红蛋白、血小板及血浆游离血红蛋白水平比较差异均无明显统计学意义($P>0.05$)。而实验组过滤时间短于对照组,血液回收率高于对照组,且24小时内溶血比例明显低于对照组($P<0.05$)。**结论:**常规法与湿润滤盘法均能达到去白细胞血液标准,但湿润滤盘法较常规法能有效的降低过滤时间、增加血液回收率,减少去白细胞悬浮红细胞因溶血造成的血液不合格率,值得临床推广应用。

关键词:全血过滤方法;去白细胞血液制备;临床对照研究

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Clinical Comparative Study of Different Whole Blood Filtration Methods for Blood Preparation of Leukocytes*

OU Fei¹, GONG Yue-ying², ZHANG Li¹, MA Lan¹, SONG Yan¹

(1 Qinghai Blood Center, Xining, Qinghai, 810000, China; 2 Qinghai Provincial People's Hospital, Xining, Qinghai, 810000, China)

ABSTRACT Objective: To investigate the effect of different whole blood filtration methods on the blood preparation of leukocytes.

Methods: Two kinds of whole blood filtration methods were used for comparison. The control group adopted the routine method. After mixing the whole blood, the whole blood was mixed directly with the leucocyte filter. The test group adopted the wet filter plate method. The blood collection was finished after mixing, and the upper serum 10~20 mL was used to moisten the filter plate and then mixed with the white cell filter. The filtration time, blood recovery rate, blood indexes before and after filtration and the incidence of hemolysis within 24 hours were compared between the two groups. **Results:** There was no significant difference in the levels of white blood cells, red blood cells, hemoglobin, platelets and plasma free hemoglobin between the two groups before and after whole blood filtration ($P>0.05$). While the filtration time of test group was shorter than the control group, the blood recovery rate was higher than that of the control group, and the percentage of hemolysis in the 24 hours was significantly lower than that in the control group ($P<0.05$). **Conclusion:** Both the conventional method and the humid filter disk method can achieve the standard of leukocyte blood, but the wet filter disc method can reduce the filtration time, increase the blood recovery rate and reduce the blood disqualification rate caused by the hemolysis of leucocyte suspended red cell, which is worthy of clinical application.

Key words: Whole blood filtration method; Leukocyte-free blood preparation; Clinical control study

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前言

随着社会医疗技术不断发展,很多患者可通过血液成分输注延长生命及提高生命质量,也因此临床上对输血的安全性和有效性的要求日益提高^[1,2]。白细胞是血液组成的重要成分之一,也是人体免疫防御系统的重要组成部分,其中的淋巴细胞具有较强的免疫活性,不仅是导致非溶血性发热反应的元凶,同时也是经血传播病原体在血液中的主要载体^[3]。输入含有白细胞的全血或血液成分引起的非溶血性发热反应、寒颤、HLA

同种免疫等副反应、病毒传播等均对医疗安全造成严重威胁,并危及患者生命健康^[4,5]。

众多临床试验证实去除全血中的白细胞后输注血液制品,能有效防止输血相关副反应的发生,提高临床用血安全性^[6-8]。此外,在全血过滤过程中,血站工作人员按照操作标准规程执行,轻柔操作,充分混匀全血,不用力挤压滴管或血袋,避免造成红细胞挤压破裂而溶血反应,上述操作因素对合格血液制品的制备也非常重要。但临床工作中,如何更有效地去除全血中的白细胞,提高血液制品的品质是血液制备工作的重点及难

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作者简介:欧斐(1975-),女,本科,副主任技师,研究方向:输血技术,电话:18997201979, E-mail:oufei_19750610@163.com

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点^[9,10]。鉴于此,本研究采用两种不同的全血过滤方法,比较其去白细胞血液制备方法所用过滤时间、血液回收率、过滤前后血液指标情况及 24 小时内溶血情况,旨在探讨提高去白细胞血液制品的方法,为临床用血提供可靠保证,现报道如下。

1 材料与方法

1.1 仪器设备和耗材

一次性使用去白细胞塑料采血袋(即采即滤型,四川南格尔);超大容量低温离心机(Thermo 12BP);SE250 热合机(韩国森通);低温操作柜(上海诺骊);储血冰箱(中国海尔)。

1.2 去白细胞血液制备

1.2.1 标本 随机选取 2017~2018 年采集的无偿献血者 1200 份人全血,每份 400 mL,采集后的全血立即置于(4±2)℃ 储血冰箱中保存。

1.2.2 对照组 将 600 袋全血制备时,轻轻混匀血液后直接进行过滤,滴速为(200~250)滴/分,防止滴速过快而导致直线过滤的出现。待过滤完成后,弃去全血袋和滤盘,之后根据血站技术操作规程(2015 版)离心进行分离制备。操作过程中应注意:过滤前应保证操作轻柔,充分混匀全血,一旦发生滤过不畅时要检查管路是否通畅,不能用力挤压滴管或血袋,避免造成红细胞挤压破裂而溶血反应。

1.2.3 实验组 将 600 袋全血采集后立即放于(2~6)℃ 冰箱中储存,将血袋垂直放置,待血浆与红细胞之间形成清晰界面,进

行分离。过滤前,轻轻挤压血袋,利用 10~20 mL 上层血清湿润滤盘,滤盘膜充分浸润后,再混匀全血与白细胞滤器连接后进行过滤,后续过滤、离心、分离制备过程同上。操作注意点同对照组,即滤前应保证操作轻柔,充分混匀全血,避免用力挤压血袋。

1.3 检测指标及方法

比较两组制备方法过滤前后血液指标情况、所用过滤时间、血液回收率及 24 小时内溶血情况:(1) 血液指标:取过滤前后的全血或悬浮红细胞 1 mL,红细胞(RBC)和血红蛋白(Hb)使用全自动血细胞计数仪测定,白细胞(WBC)和血小板(PLT)用细胞计数板测定,计数 2 个计数池,血浆游离血红蛋白(FHb)采用邻甲联苯胺法测定;(2) 过滤时间、血液回收率:计算过滤所用时间(分)及血液回收率;(3) 24 小时内溶血情况:全血离心制备后 24 小时内,血浆颜色呈浅红、粉红、深红均可判定为溶血。

1.4 统计学处理

应用 SPSS 22.0 软件进行数据分析,计量资料以($\bar{x} \pm s$)表示,组间比较行 t 检验;计数资料以率表示,组间比较行卡方检验,以 $P < 0.05$ 差异有统计学意义。

2 结果

2.1 两组过滤前后血液指标比较

两组全血过滤方法过滤前后 WBC、RBC、Hb、PLT 及 FHb 比较差异均无明显统计学意义($P > 0.05$)。见表 1。

表 1 两组过滤前后血液指标比较($\bar{x} \pm s$)

Table 1 Comparison of the blood indexes between two groups before and after filtration($\bar{x} \pm s$)

Index	Control group		Experimental group	
	Pre filtration	Post filtration	Pre filtration	Post filtration
WBC($\times 10^9/L$)	5.18± 1.12	0.0017± 0.0012	4.79± 1.02	0.0014± 0.0011
RBC($\times 10^{12}/L$)	4.71± 0.37	4.85± 0.39	4.69± 0.31	4.68± 0.32
Hb(g/L)	118.32± 14.13	128.4± 12.96	120.32± 12.01	129.11± 10.32
PLT($\times 10^9/L$)	170.21± 33.01	23.13± 5.21	168.31± 29.42	22.68± 4.94
FHb(mg/L)	129.43± 3.7	132.11± 4.22	128.72± 3.21	130.31± 4.72

2.2 两组过滤法的过滤时间、血液回收率比较

实验组全血过滤方法所用过滤时间短于对照组、血液回收

率高于对照组,差异具有统计学意义($P < 0.05$)。见表 2。

表 2 两组过滤时间、血液回收率比较($\bar{x} \pm s$)

Table 2 Comparison of the filtration time and blood recovery between two groups($\bar{x} \pm s$)

Groups	Filtration time(min)	Blood recovery rate(%)
Control group	13± 2.3	86.63± 2.56
Experimental group	9± 2.4*	91.82± 2.49*

Note: compared with the control group, * $P < 0.05$.

2.3 两组 24 小时内溶血情况比较

实验组 24 小时内溶血比例 (0.16%) 明显低于对照组 (0.83%),差异有统计学差异($P < 0.05$)。见表 3。

3 讨论

血液中含有多种有形成分,主要包括红细胞、白细胞、血小

板。血液中的白细胞可引起多种输血不良反应,如过敏、非溶血性发热反应、病毒传播(如艾滋病^[11]、肝炎^[12])等,严重威胁医疗技术安全及患者的生命健康。既往研究显示非溶血性发热的发生与受体体内既往是否产生白细胞抗体、输注血液成分白细胞、血小板的“清洁程度”密切相关。除此以外,全血在储存时,其中的白细胞以及白细胞死亡崩解后能够分泌一些生物活性

表 3 两组 24 小时内溶血情况比较

Table 3 Comparison of the hemolysis between two groups within 24 hours

Groups	Specimen bag (bag)	Hemolysis number (bag)	Hemolysis rate (%)
Control group	600	5	0.83
Experimental group	600	1	0.16*

Note: compared with the control group, *P<0.05.

分子(如白介素、肿瘤坏死因子),这些生物活性分子会导致输血时发生过敏等不良反应^[13,14]。若将全血中的白细胞过滤掉,则可在一定程度上减少不良反应的发生。在去白细胞过滤器的临床疗效得到充分肯定后,很多国家已完全实行去白细胞血液和血液成分输注,在非常大的程度降低了白细胞相关输血反应和输血相关的病毒传染的发生率^[15]。目前,我国中心血站采用的血站型过滤器过滤法去除全血中的白细胞,是通过筛分和粘附来去除白细胞的。同时,血小板在过滤过程中也能够粘附在滤器滤膜上,促进白细胞的进一步粘附,大大提高过滤效率,改善血液制品的品质。通过将血液中的白细胞滤除这一操作,大大减少了血液中白细胞、血小板^[16]以及蓄积的各种氧化代谢产物^[17],进而减少了上述成分对红细胞以及受血者免疫系统的损害,降低了非溶血性发热反应、病毒传染性疾病的发生,提高了临床用血的安全性^[18,19]。同时,我国采用统一严格执行操作标准规程,操作人员经过培训上岗后熟练掌握操作技术,这对减少白细胞过滤过程中的人为误差,提高滤过治疗是非常有必要的。全血过滤后白细胞残留量 $<2.5 \times 10^6/L$,红细胞回收率 $\geq 85\%$,FHB $<530\text{ mg/L}$ 是我国规定的去白细胞滤器的标准。本研究中,两种方法过滤后的血液质量均达到上述标准,具有高白细胞清除率及红细胞回收率,临床均可应用。

过滤过程中多种因素均会影响白细胞过滤效果,如红细胞采集后贮存会的温度会影响血液过滤的效果,因此临床上常将血液置于 $2\sim 6^\circ\text{C}$ 冰箱中保存^[20]。此外,红细胞贮存时间也将影响过滤效果^[21-23],红细胞贮存的时间越长,越容易发生溶血反应^[21]。一般认为,过滤前后血红蛋白含量的变化和过滤时丢失的血液量决定了血液的回收率^[24],本研究结果显示湿润滤盘法滤除白细胞的时间明显少于常规法,可能是因为滤过率阻力降低、过滤时较通畅所致,且过滤后血液回收率高;白细胞滤器所用材料为聚酯纤维,当血液流过时部分血液成分被吸附,血清湿润滤盘后丢失的血液量减少,因而血液回收率更高^[25]。

白细胞过滤时,所采用的方法不同,溶血反应发生率也就不同^[26,27]。本研究结果显示常规法比湿润滤盘法过滤后血液制品的溶血发生率更高,原因在于通常所采用的白细胞过滤器的滤芯材料为聚酯纤维无纺布,干燥滤芯在血液经过滤盘时会大量吸收水分,细胞膜稳定性和变形能力因失水而降低,进而粘度升高而破裂,红细胞中的血红蛋白溢出导致溶血反应的发生^[25,28]。湿润滤盘法是在过滤前用少量血清浸润滤盘,避免红细胞脱水,减少红细胞通过滤盘时的机械阻力,减少溶血的发生。另有研究显示红细胞过滤后发生溶血的可能性随保存时间的延长而增大^[29,30]。本研究中,血液储存时间在24 h内,采用湿润滤盘法过滤白细胞能有效降低红细胞溶血的发生,提高了血液质量,减少了血液的浪费。

此外,在全血过滤过程中,操作的准确、稳定性对血液成分的质量也影响巨大,血站工作人员严格执行操作标准规程,熟练掌握操作技术,减少白细胞过滤过程中的人为误差,对提高滤过治疗是至关重要的。过滤前应保证充分混匀全血,操作轻柔,以免混合不均匀造成过滤不通畅而影响白细胞滤过的效果,一旦发生滤过不畅时要检查管路是否通畅,不能用力挤压滴管或血袋,避免造成红细胞挤压破裂而溶血反应。

综上所述,常规法与湿润滤盘法均能达到去白细胞血液标准,但湿润滤盘法较常规法更能有效的降低过滤时间、增加血液回收率,减少去白细胞悬浮红细胞因溶血造成的血液不合格率,值得临床推广应用。

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