Observational study of blood complement levels of components before infusion

Zhi Han[1], Yujie Han[2], Haihong Zhang[3, *]

[1]Wuwei Central Blood Station, Wuwei, China, 733000

[2]Central University for Nationalities, Beijing, China, 100081

[3]Gansu Province Red Cross Blood Center, Lanzhou, China, 730046

* Corresponding Author Email: 420438256@qq.com

Abstract. To investigate the changes in complement content in component blood before clinical transfusion. According to different preparation processes, storage temperatures and time periods, 180 specimens of plasma from the same source of 20 people were left at different stages of the preparation process. Homologous specimens were concentrated in the same batch of experiments to observe the changes in complement C3 and C4 content under different storage temperature and process stage grouping conditions. Using the same specimen to detect C3 and C4 content, the detection results of homologous specimens were sorted into nine groups according to different storage temperatures and preparation process stages for comparison.and there is no significant difference between simple -40°C and -60°C storage at different temperatures. Storage at 4°C for 7 days can significantly reduce complement content. Virus inactivation preparation process can significantly reduce complement content. Repeated freezing and thawing during the processing of frozen plasma can change complement content. Correlation analysis of complement C3 and C4 content determination experiments showed that the changes of the two under different conditions such as temperature, preparation process and storage time were significantly positively correlated. The storage temperature and storage time of frozen plasma, repeated freezing and thawing, centrifugal concentration and light inactivation can affect the complement content.

Keywords: Complement, Plasma, Temperature, Content.

1. Introduction

Complement is a group of globulin molecules normally present in blood or body fluids that are involved in immune effects and can be enzymatically active. The current transfusion industry standards do not specify requirements for complement content. C3 serum has the highest content in each complement component, and is functionally the core molecule of the complement system. Complement accounts for 10% of the total serum globulin, the content is relatively stable, and antigenic system has nothing to do with stimulation, so it does not increase with the establishment and enhancement of body-specific immunity[1-2], detection of human plasma complement levels, can be used as an auxiliary diagnostic evidence to understand the condition of certain diseases. Because clinical treatment often requires transfusion of blood products, and the consequent input of complement components into the human body in the host body content and role of the study is not common[3].

Under normal physiological conditions, the majority of complement intrinsic components exist in a non-activated form, and a series of cascade reactions occur after the action of a certain activator, in which various complement intrinsic components are activated sequentially, ultimately producing a cytolytic effect. At the same time, complement can also mediate pathological injuries, such as causing allergic reactions, intravascular hemolysis of imported red blood cells, lysis of red blood cells or white blood cells and platelets, activation of platelets, etc.. Therefore, complement molecules have a very important role in transfusion medicine[4-5].

The complement system is known to date to consist of more than 20 components. The highest levels in serum are found in C3, followed by C4, S proteins and factor H. In order to observe the changes in the complement content of component blood before entering the human body, the author

separated plasma samples from -60℃, -40℃, 4℃, and 22℃ at different preservation temperatures and stages of the preparation process from 6h after blood collection, and detected the content of complement C3 and C4 of several common blood products in the region at different stages of preservation, with a view to reflecting the changes in the content of protein components, such as complement, of component blood prior to clinical transfusion, and accumulating data for the evidence-based blood transfusion medicine[6].

2. Materials and methods

2.1. Subjects

Randomly selected from this site during the period August 2022—October 2022 from the blood of unpaid blood donors separated from the 20-person portion of ordinary plasma, and 20-person portion of fresh frozen plasma in the preparation process at different stages of the respective homologous specimens retained in a total of 180 copies of the unpaid blood donors are in line with the "Blood Donor Health Examination Requirements", Determination of complement C3 and C4 content of several common component blood products at different preparation stages and temperatures[7].

2.2. Experimental methods

Complement C3 and C4 were detected by immunoturbidimetric assay.

(1)Equipment Hitachi 008 AS automatic biochemistry analyzer

(2) reagents Complement C3 and C4 were used with lot number 220817102 Meikang Bio kit, and the QC was Langdao HN1530 with QC lot number 1376UN.

2.3. Experimental Grouping

According to the different preparation process, preservation temperature and time period, 20 human plasma from non-remunerated blood donors in this site at different stages of the preparation process retained a total of 180 copies of their respective homologous specimens, concentrated in the same batch of experimental testing, observation of different preservation temperature and process stage grouping conditions under the changes in the content of complement C3, C4, the results of the experiments are divided into nine groups, the following numbers in parentheses (i) to (ix) for the test results of the grouping number[8].

(1) Blood was centrifuged to separate cells and plasma within 6 h after collection, and the first batch of specimens were retained with plasma snap-frozen and stored at -40°C and -60°C, respectively, to represent the original plasma at -40°C (i) and the original plasma at -60°C (ii).

(2) Ordinary plasma plus methylene blue 6 ℃ light for virus inactivation, the second batch of specimens were retained before quick-freezing, along with the plasma were stored at -40 ℃ and -60 ℃, respectively, and stored at 4 ℃ for 7 days before the test was set up to simulate the whole blood was stored at 4 ℃ in the state of 7 days after the viral inactivation process of the ordinary inactivation of the plasma at -40 ℃ (iii) and ordinary inactivation of the plasma at -60 ℃ (iv).

(3) Fresh frozen plasma after separation and cold precipitation, plus methylene blue at 6 ℃ light virus inactivation, quick-freezing before retaining the third batch of specimens, along with the plasma were stored at -40 ℃ and -60 ℃, respectively, representing the fresh frozen plasma after the separation and cold precipitation, viral inactivation process of the new inactivation of the plasma at -40 ℃ (v) and -60 ℃ new inactivation of the plasma, in which the new inactivated plasma of -60 ℃ in the first 7 days of the assay was placed at 4 ℃, representing the new inactivated plasma of the new inactivation plasma of the plasma after two processes and preserved for 7 days at 4 ℃ (vi).

(4) Fresh frozen plasma was stored for 30 days before being thawed in a 6°C water bath to separate the cold precipitate by centrifugation, and the coupling bag connecting line was heat-sealed and retained for the fourth batch of specimens before being disconnected, and the accompanying plasma

was stored at -40°C and -60°C to represent the cold precipitate (vii) and cold precipitate (viii) at -60°C, respectively, separated from cold preparations by centrifugation.

(5) Fresh frozen plasma was cold-precipitated at -60°C after the separation cold-precipitation process (viii) The specimen was divided into two, and the divided portion was placed at 22°C for 48 h of shock storage prior to the assay to simulate 48 h of platelet concentration (ix).

2.4. Statistical methods

SPSS 26.0 statistical software was used to process the data, and the measurement information was expressed as ($\bar{x}\pm s$), and the wilcoxon signed rank test in the rank sum test was used, and the difference was considered to be statistically significant at $P\leq0.05$, and the correlation comparison between the groups was performed by Spearman correlation analysis, and the difference was considered to be statistically significant at $P\leq0.05$[9-10].

3. Findings

Table 1. Comparison of Complement C3 and C4 Content Assay Results(n=20)

clusters	1	2	3	4
C3	0.76±0.12	0.82±0.15	0.72±0.13	0.77±0.13
p	①0.9941	②0.1333	③0.2406	④0.0470
C4	0.24±0.05	0.25±0.05	0.22±0.04	0.23±0.04
p	①0.9571	②0.0273	③0.0251	④0.0426
clusters	6	7	8	9
C3	0.76±0.16	0.93±0.16	0.80±0.11	1.04±0.17
p	⑥0.9999	⑦0.4186	⑧0.9979	⑨1.0000
C4	0.22±0.04	0.27±0.04	0.25±0.04	0.31±0.06
p	⑥0.9987	⑦0.5640	⑧0.9968	⑨0.9997

Note: Rank sum test p-value: ① comparison of group 1 and group 2; ② comparison of group 1 and group 3; ③ comparison of group 2 and group 4; ④ comparison of group 1 and group 5; ⑤ comparison of group 2 and group 6; ⑥ comparison of group 1 and group 7; ⑦ comparison of group 2 and group 8; ⑧ comparison of group 8 and group 9; ⑨ comparison of group 2 and group 9.

3.1. Comparison of Complement C3 and C4 Content Test Results

The same specimen was used to test C3 and C4 content, and the results of homologous samples were organized into nine groups for comparison according to the grouping conditions, as shown in Table 1.

(1) Comparison of plasma complement C3 content at different stages Observe the results of complement content assay under different preservation temperatures and time period grouping conditions. (i) There was no significant change in the complement C3 content of plasma from raw plasma preserved at temperatures of -40℃ and -60℃; (ii) There was no significant change in the complement C3 content of plain inactivated plasma preserved at -40℃ versus that of simulated whole blood preserved at 4℃ for 7 days; (iii) There was no significant change in the complement C3 content of raw plasma preserved at -60℃ versus that of plain inactivated plasma preserved at simulated whole blood preserved at 4℃ for 7 days; (iv) There was no significant change in the complement C3 content of raw fresh frozen plasma preserved at -40 ℃ of the original fresh frozen plasma in the preparation of new inactivated plasma after a significant change in the content of complement C3, preservation time and preservation temperature after preparation did not change, proving that the preparation of cold precipitation preparation process there are factors that can significantly change the content of complement C3; ⑤ preserved at -60 ℃ of the original fresh frozen plasma and the preservation of new inactivated plasma 4 ℃ the difference between the content of complement C3, preservation time

did not change, and the preservation temperature of the prepared There is a change in the preservation temperature, proving that the preparation of cold precipitation after the preparation of new inactivated plasma preparation process, and the preservation of the preparation of the temperature of the two in the existence of significant changes in the content of complement C3 factors; (6) preserved at -40 ℃ of the original fresh plasma and -40 ℃ of the new inactivated plasma complement C3 content did not change significantly; (7) preserved at -60 ℃ of the original fresh plasma and -40 ℃ concentration of cold precipitation complement C3 content did not have a significant difference; ⑧ there was no significant change in the complement C3 content of cold precipitate and platelets preserved at -60°C, i.e., there was no significant change in the complement C3 content between concentrated cold precipitate and platelet-concentrated plasma; ⑨ there was no significant change in the complement C3 content between pristine fresh plasma and simulated platelet-concentrated plasma preserved at -60°C.

(2) Comparison of complement C4 content of plasma at different stages Observed the results of complement content assay under different preservation temperatures and time period grouping conditions, which were basically consistent with the conclusion of the above comparison of complement C3 content. There was no significant change in the complement C4 content of the original fresh plasma stored at -40℃ and -60℃; ② There was a significant change in the complement C4 content of the original fresh plasma stored at -40℃ and the simulated whole blood stored at 4℃ for 7 days, and there was no change in the preservation time and the preservation temperature before preparation, which proves that there are influencing factors that can significantly change the complement C4 content of the preparation process of preparing spectral plasma and preservation temperature after preparation; ③ There was a significant change in the complement C4 content of the original fresh plasma stored at -60℃ and simulated whole blood stored at 4℃ for 7 days. There was a significant change in the complement C4 content of the original new plasma stored at -60℃ and the simulated whole blood stored at 4℃ for 7 days, and there was no change in the preservation time and preservation temperature before preparation, which proved that there were influencing factors that could significantly change the complement C4 content in both the preparation process of the prepared spectrum extirpated plasma and the preservation temperature after preparation; ④There was a significant change in the complement C4 content of the original new plasma and the new extirpated plasma stored at -40℃, and there was no change in the preservation time and temperature. There is a significant difference in the complement C4 content between the original new plasma stored at -40℃ and the new inactivated plasma stored at -40℃, with no change in the preservation time and temperature, which proves that the process of preparing virus-inactivated plasma can significantly change the complement C4 content. factors; (6) the original new plasma stored at -40 ℃ and -40 ℃ cold precipitation complement C4 content did not change significantly; (7) the original new plasma stored at -60 ℃ and -60 ℃ concentration of cold precipitation complement C4 content is not significantly different; (8) stored at -60 ℃ concentration of cold precipitation and simulated platelet concentrate between the content of complement C4 did not change significantly; (9) stored at -60 ℃ the original new plasma and simulated platelet concentrate There was no significant change in complement C4 content between the original fresh plasma stored at -60°C and simulated platelet concentrate.

3.2. Correlation analysis between complement C3 and complement C4

According to the results of Spearman's correlation analysis, there was a significant positive correlation between complement C3 and complement C4 ($P<0.05$), i.e., the content of complement C3 and C4 varied consistently under the conditions of consistency of the variables of preservation temperature, time period, and preparation process, as shown in Table 2.

Table 2. Spielman-related analysis

	complement C3	complement C4
complement C3	1.000	0.894**
complement C4	0.894**	1.000

*** $p<0.01$, ** $p<0.05$, * $p<0.1$

4. Discussion

The current transfusion industry standards do not specify requirements for complement content. C3 serum has the highest content in each complement component and is functionally the core molecule of the complement system. Complement accounts for 10% of the total serum globulin, the content is relatively stable, and antigenic system has nothing to do with stimulation, so it does not increase with the establishment and enhancement of body-specific immunity, detection of human plasma complement levels, can be used as an auxiliary diagnostic evidence to understand the condition of certain diseases. Because clinical treatment often requires transfusion of blood products, and the accompanying input of complement components into the human body in the host body content and role of the study is not common.

Complement activity is not very stable, can be inactivated in 30 minutes by heating at 56℃. After blood leaves the human body, the complement content in it varies at different temperatures and with the change of preservation time. In order to truly reflect the changes of complement components under different preservation and preparation process conditions and to ensure the comparability of the test data, homologous plasma was used for the test specimens, and the specimens retained from all stages of preparation and preservation were in the same preservation conditions as the blood component products, i.e., nine groups of test specimens were homologous.

Limited to the conditions, did not do plasma complement activity and stability research, this experiment only detects plasma products in different preservation temperature and preparation process stage of the complement C3, C4 content, through the comparison of the original plasma preserved at -40 ℃ and -60 ℃ under the conditions of the complement of C3, C4 content differences are not significant; preserved at -40 ℃ and -60 ℃ of the original plasma and the ordinary inactivated plasma Complement C3 content of the original plasma stored at -40 ℃ and -60 ℃ and ordinary inactivated plasma changes are not significant, C4 content changes are significant, inconsistency is analyzed as a result of insufficient sample size, the trend suggests that the 4 ℃ preservation temperature and viral inactivation process can reduce the content of complement; preservation of the original plasma stored at -40 ℃ and -40 ℃ of the new inactivated plasma complement C3, C4 content have a significant difference, proving that the plasma freezing and thawing, light inactivation and other preparatory processes can significantly change the complement C3, C4 The contents of complement C3 and C4 in the original fresh plasma stored at -60℃ and the newly inactivated plasma stored at 4℃ for 7 days after preparation were significantly different, proving that the preparation process of cold precipitation such as 4℃ storage temperature, plasma freezing and thawing, and light inactivation can significantly change the contents of complement C3 and C4; the contents of complement C3 and C4 in the original fresh plasma and the cold precipitated plasma at -40℃ and -60℃ were not significant, analyzed as low temperature, and analyzed as low temperature, and analyzed as low temperature, and analyzed as low temperature, and analyzed as low temperature, and analyzed as low temperature and low temperature, and analyzed as low temperature and low temperature. were not significant, analyzed as low-temperature centrifugation led to plasma complement and other proteins concentration; stored at -60 ℃ concentration of cold precipitation and 22 ℃ preservation of 48h simulated concentration of platelet complement C3, C4 content reduction was not significant, and there is a tendency to increase, analyzed as 22 ℃ shock preservation process to make the original low-temperature centrifugation precipitation of concentrated plasma complement and uniformly dissolved in the plasma.

In summary, the changes in complement content were not significant when preserved at different temperatures of -40°C and -60°C alone; preservation at 4°C for 7 days was able to significantly reduce the complement content; and preservation at 22°C at room temperature did not show any significance in 48h. It has been reported that low temperature is preferable for preserving complement, and the above conclusions are consistent with the reported findings. The experimental correlation analysis for the determination of complement C3 and C4 content showed a significant positive correlation between the changes under the same conditions of temperature, preparation process and storage time.

5. Conclusion

This study found that some factors can affect the complement content, frozen plasma repeated freezing and thawing, centrifugal concentration, light inactivation and other factors can reduce the plasma complement content. To further grasp the plasma complement content of each component before transfusion, to ensure the quality of blood to provide a reference basis for the observation of plasma complement content study grouping, sample retention to provide ideas, to consider whether it can be helpful for clinical evaluation of the prognosis of the patient's condition after transfusion, and if the number of samples to increase the number of samples will be further proof of the above conclusions.

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