A proposed predictive mathematical model for efficient T-cell collection by leukapheresis for manufacturing chimeric antigen receptor T cells

Manufacturing chimeric antigen receptor T cells (CAR T cells) requires collection of $CD3^+$ lymphocytes through mononuclear cell (MNC) leukapheresis. MNC leukapheresis for autologous CAR T cells manufacturing in patients with relapsed/refractory leukemia and lymphoma who have undergone multiple lines of chemotherapies creates various challenges. Firstly, patients' leukopenia and lymphopenia make the red blood cell-white blood cell interface in the apheresis machine difficult to be established.[1] Secondly, patients are subjected to long durations of leukaphereses with large volumes of blood processed in order to obtain sufficient $CD3^+$ lymphocytes. Korell *et al.* advocated processing a minimal of 12-15 liters of blood in order to harvest sufficient number of $CD3^+$ lymphocytes for CAR T-cell manufacturing.[2] Patients often have suboptimal performance status and physical reserve to tolerate such long leukaphereses. Lastly, patients with relapsed/refractory leukemia and lymphoma usually have a small window period for successful leukapheresis when they are free of infections and their physical states are able to tolerate the leukapheresis. The current practices of MNC leukapheresis rely on processing a large volume of blood, which often leads to unnecessarily prolonged apheresis, wastage of manpower and hospital resources, and exposing the patients to additional risks associated with prolonged leukaphereses. Studies have reported various factors that impact the final $CD3^+$ lymphocyte yield,[3,4] and the preleukapheresis $CD3^+$ count is the one common determining factor that has been repetitively mentioned. Preleukapheresis $CD3^+$ count is difficult to be altered in patients with relapsed/refractory leukemia and lymphoma. Hence, patients with lower preleukapheresis $CD3^+$ counts require larger volumes of blood processed during leukaphereses to meet a target yield and *vice versa*. A formula that can determine the required processed blood volume for patients based on their preleukapheresis $CD3^+$ count to meet the target $CD3^+$ lymphocyte yield will help to improve the efficiency of leukaphereses. In this study, we tried to understand the dynamics of the $CD3^+$ lymphocyte collection through MNC leukapheresis and derive a predictive mathematical model using preleukapheresis $CD3^+$ count to guide the required blood volume to be processed.

We have included three sets of data in this study. The first set of data is from 12 MNC leukaphereses for CAR T-cell manufacturing at the Singapore General Hospital (SGH), Department of Hematology from May 2020 to June 2021. The second set of data consists of five MNC leukaphereses performed at a different institute, the National University Hospital Singapore (NUH). This set of data was used to verify the consistency of the findings at a different institute. The third set of data consists of another six MNC leukaphereses for CAR T-cell manufacturing from June 2021 to April 2022 at SGH. This set of data was used to verify if the proposed mathematical equation derived from past data is applicable for future leukaphereses at the same institute. All patients had relapsed/refractory diffuse large B-cell lymphoma where majority had at least three lines of therapies. MNC leukaphereses were performed at least 1 month from the last cycle of chemotherapy. This study was approved by the Institutional Review Board and Ethics Committee of Singapore General Hospital. Consent were provided by all patients. All leukaphereses were performed using the "Terumo" Spectra Optia Apheresis system version 11.3 with continuous mononuclear cell collection (cMNC) protocol. Important parameters collected during the MNC leukaphereses were: preleukapheresis $CD3^+$ lymphocyte count (denoted as $C_{preleukapheresis}$, 10^9 cells/L), the total blood volume processed (denoted as V_T, L), the total body blood volume for each patient (denoted as V_B, L), the total amount of $CD3^+$ lymphocyte yielded (denoted as T, 10^9 cells).

The 12 leukaphereses at SGH between May 2020 and June 2021 were analyzed to derive the mathematical model. Table 1 summarizes the basic demographic, preleukapheresis laboratory data, and collection data of the 12 leukaphereses.

Previous studies,[5,6] described $CD3^+$ lymphocytes collection efficiency (CE) as: $CE=T/(C_{preleukapheresis}*V_T)$. The calculated CE of the 12 leukaphereses performed at SGH varied widely between 21.4-95.1% (mean 67.4%; standard deviation 20.5%). Similarly, CE of the five leukaphereses performed at NUH also varied widely between 26.3-75.0% (mean 53.7%; standard deviation 17.5%). Finding a representative CE for an institute may not be practical and may result in erroneous estimation of blood volume to be processed. The correlation between T and $C_{preleukapheresis}*V_T$ in above equation only had an R^2 of 0.75 for the 12 SGH leukaphereses. We think the reason for the wide variation of CE observed is due to the constant change of "real-time" circulating $CD3^+$ lymphocytes concentration during leukapheresis, because $CD3^+$ lymphocytes are constantly re-

Table 1. Summary of basic demographic, preleukapheresis laboratory data, and collection data for the 12 leukaphereses at the Singapore General Hospital from May 2020 to June 2021.

Patients N=11 Leukaphereses N=12*	Mean	Range	Standard deviation
Patient demographics			
Female sex, N	10 (91%)	-	-
Age in years	54	19-73	16
Height, cm	160.7	148-179	8.3
Weight, kg	56.6	40-85.1	12.5
BMI	22.0	16.3-30.0	4.0
Preleukapheresis laboratory data			
Hemoglobin level, g/dL	10.28	7.8-12.5	1.41
Hematocrit, %	30.63	24.3-35.2	3.60
Lymphocyte count $x10^9$/L	0.71	0.23-1.67	0.44
Prepheresis $CD3^+$ lymphocyte count $x10^9$/L	0.57	0.20-1.49	0.37
Leukapheresis data			
Total body blood volume, L	3.57	2.80-5.45	0.68
Total blood volume processed, L	10.19	5.52-16.78	2.67
Total collection time, minute	319	117-410	59
Total mononuclear cell collected $x10^9$	12.25	5.75-19.65	4.90
Total $CD3^+$ lymphocyte collected $x10^9$	3.57	0.67-7.66	1.97

*Note: the 12 leukaphereses were performed on 11 patients as 1 patient required a second collection. All patients had relapsed/refractory B-cell lymphoma and had previously been treated with at least 3 lines of systemic chemotherapies. BMI: body mass index.

moved from the peripheral blood during leukapheresis. The rate of $CD3^+$ lymphocyte collection gradually slows down as more blood is being processed. Assuming a constant CE assumes a linear relationship between V_T and T, i.e., doubling V_T can result in doubling of T. This is unrealistic and will result in overestimation of the $CD3^+$ lymphocyte yield.

In order to account for the constant change in the "real-time" circulating $CD3^+$ lymphocytes concentration, we made two assumptions: first the total amount of $CD3^+$ lymphocytes circulating in the peripheral blood is not replenished from the extravascular space during MNC leukapheresis; secondly the apheresis machine removes a fraction (η) of the $CD3^+$ lymphocyte from the blood that is fed to the machine ($0< \eta <1$). Figure 1 is a simplified illustration of the apheresis process, where t represents the duration of leukapheresis. The change of the amount of $CD3^+$ lymphocytes in the patient's body $d(V_B*C_1)$ equals to the amount of $CD3^+$ lymphocyte removed by the apheresis machine $(C_2-C_1)*v*dt$; therefore: $d(V_B*C_1)/dt=(C_2-C_1)*v$. By assumption 2, $C_2-C_1=-\eta*C_1$. Solving the differential equation stated above results in: $C_1=e\text{^}constant*e\text{^}(-\eta*v*t/V_B)$. At $t=0$, C_1 equals the preleukapheresis $CD3^+$ lymphocyte concentration, $C_{preleukapheresis}$; hence: $C_1=C_{preleukapheresis}*e\text{^}(-\eta*v*t/V_B)$. $v*t$ equals the total volume of blood processed, V_T; hence: $C_1=C_{preleukapheresis}*e\text{^}(-\eta*V_T/V_B)$. The total $CD3^+$ lymphocyte collected can therefore be expressed as: $T=(C_{preleukapheresis}-C_1)*V_B=C_{preleukapheresis}*V_B*(1-e\text{^}(-\eta*V_T/V_B))$. This equation suggests that the maximum T from one leukapheresis is $C_{preleukapheresis}*V_B$, which is the total amount of $CD3^+$ lymphocyte in the blood prior to leukapheresis. However, ten of the 12 leukaphereses performed at SGH between May 2020 and June 2021 were able to obtain more $CD3^+$ lymphocytes than the total amount of $CD3^+$ lymphocytes estimated in the blood prior to leukaphereses. Similar findings were observed in the five leukaphereses at NUH, where four of the five leukaphereses were able to obtain more $CD3^+$ lymphocytes than estimated in the blood prior to leukaphereses. These findings suggest that $CD3^+$ lymphocytes are possibly actively replenished from extravascular tissues during the leukapheresis, instead of the assumption that the total amount of $CD3^+$ lymphocytes in the peripheral blood is not replenished during MNC leukapheresis.

As discussed above, using a fixed collection efficiency assumes a linear relationship between V_T and T (as shown in Figure 2 by a dotted line), which results in an overestimation; whereas assuming an MNC, leukapheresis is un-

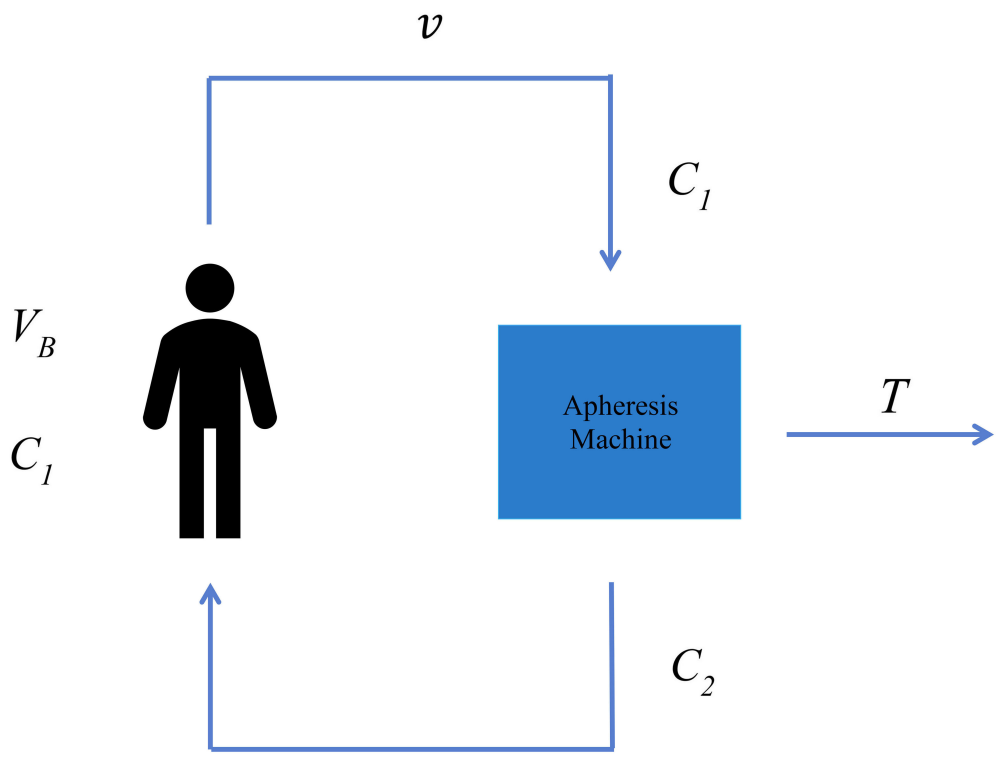

Figure 1. A simplified illustration of the leukapheresis process. C_1: concentration of CD3⁺ lymphocytes in the patient's peripheral blood at any time of leukapheresis; C_2: concentration of CD3⁺ lymphocytes in the blood leaving the apheresis machine returning to the patient; V_B: total body blood volume; v: apheresis machine blood flow rate; T: total number of CD3⁺ lymphocytes collected.

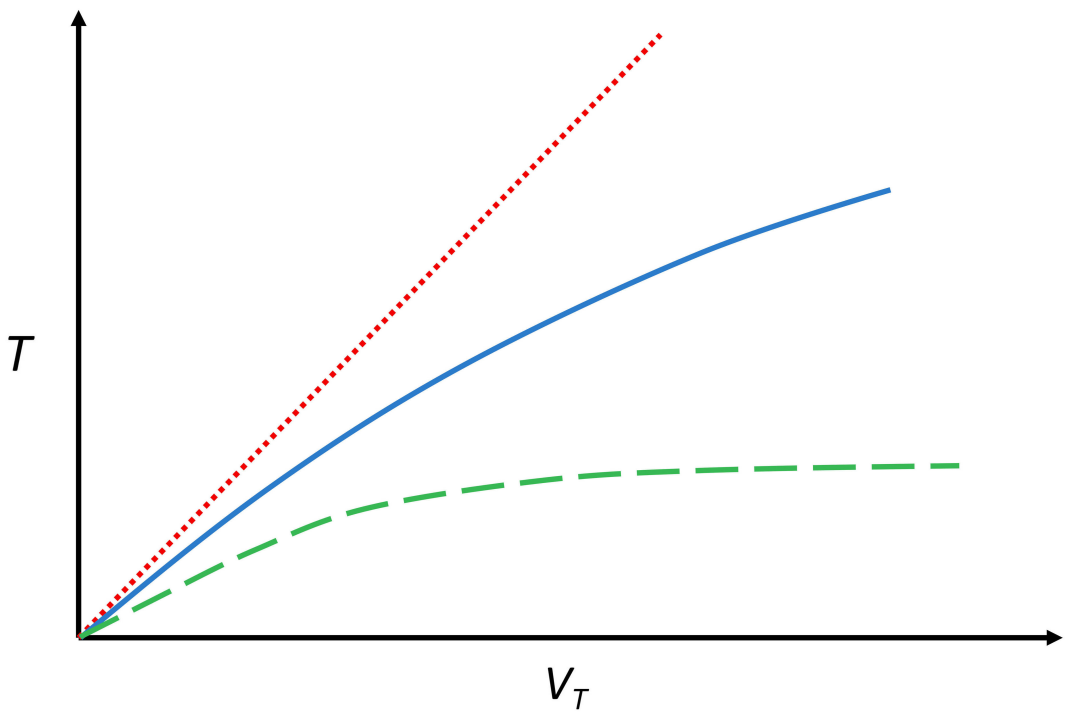

Figure 2. The visual representation of relationship between V_T and T, where V_T is the processed blood volume and T is the total CD3⁺ lymphocytes collected. The dotted line represents a linear relationship between V_T and T, which results in overestimation; the long dash line represents a plateauing relationship between V_T and T, which results in underestimation. The study postulated that the actual relationship is a curve shown in the solid line.

able to yield more than the amount of CD3⁺ lymphocytes in the blood prior to leukapheresis (as shown in Figure 2 by a long dashed line) which results in an underestimation of the actual CD3⁺ lymphocyte yield. Therefore, we postulate that the relationship is likely a curve as shown in Figure 2 by the solid line: the speed of CD3⁺ lymphocytes collected will gradually slow down, but the total number of CD3⁺ lymphocyte collected should continue to increase as leukapheresis continues. In order to describe the curve in a solid line, we used a logarithm equation to approximate it, where a and b are constants unique to each apheresis centre: $T=a*ln(C_{preleukapheresis}*V_T)+b$. Based on previous leukaphereses data, a and b can be obtained using regression line formula. Using the data from the 12 leukaphereses at SGH between May 2020 and June 2021, the equation for SGH was obtained: $T=3.588*ln(C_{preleukapheresis}*V_T)-2.006$ (R^2: 0.90). The residual standard error for this equation was 0.66. The equation obtained was tested on the six subsequent MNC leukaphereses performed at SGH between June 2021 and April 2022 and it had an R^2 value of 0.91. This showed that the equation obtained from previous data was still applicable for subsequent leukaphereses in the same center. This proposed mathematical model was also tested on data from a different institute, NUH. Constants a and b were calculated for NUH. The equation had an R^2 value of 0.97.

Previously published literature has described variables with predictive value for the CD3⁺ yield, including CD3⁺ count and hematocrit in one study[3] and CD3⁺ count, hemoglobin level, and platelet count in the other.[4] Despite some differences, the CD3⁺ count is consistently the most important variable that impacts the final CD3⁺ yield. What the current model adds to the existing ones is that it can be generalized. It requires each center to calculate their individual

constants *a* and *b* to fit the differences in the patient profiles, operator factors, and machine factors.

In summary, this study demonstrates that the yield of $CD3^+$ lymphocyte positively correlates to the preleukapheresis $CD3^+$ lymphocyte count and the volume of blood processed. The equation $T=a*ln(C_{preleukapheresis}*V_T)+b$ can be generalized to describe the $CD3^+$ lymphocyte collection through MNC leukapheresis and helps provide an estimation of the minimum blood volume to be processed to meet the $CD3^+$ lymphocyte target requirements.

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Disclosures

No conflicts of interest to disclose.

Contributions

XH contributed to the design of the study, analysis of the data and writing of the paper. GPLG, KKH and JJL contributed to the laboratory testing, data collection and drafting of the paper. SP, RS, JMLT and GX contributed to performing leukapheresis, data collection and writing of the paper. EHLC, AYLH, WYKW, YCL, YC, JKSQ, HT and CN contributed to the recruitment and assessment of the patients, guidance in the study, drafting and review of the paper. FLWIL is the principle investigator. She contributed to the conceptualisation and design of the study, guidance of the study, writing and review of the paper.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Fesnak A, Lin C, Siegel DL, Siegel DL, Maus MV. CAR-T cell therapies from the transfusion medicine perspective. Transfus Med Rev. 2016;30(3):139-145.
2. Korell F, Laier S, Sauer S, et al. Current challenges in providing good leukapheresis products for manufacturing of CAR-T cells for patients with relapsed/refractory NHL or ALL. Cells. 2020;9(5):1225.
3. O'Reilly MA, Malhi A, Cheok KPL, et al. A novel predictive algorithm to personalize autologous T-cell harvest for chimeric antigen receptor T-cell manufacture. Cytotherapy. 2023;25(3):323-329.
4. Jo T, Yoshihara S, Hada A, et al. A clinically applicable prediction model to improve T cell collection in chimeric antigen receptor T cell therapy. Transplant Cell Ther. 2022;28(7):365.e1-365.e7.
5. Allen ES, Stroncek DF, Ren J, et al. Autologous lymphapheresis for the production of chimeric antigen receptor T cells. Transfusion. 2017;57(5):1133-1141.
6. Hutt D, Bielorai B, Baturov B, et al. Feasibility of leukapheresis for CAR T-cell production in heavily pre-treated pediatric patients. Transfus Apher Sci. 2020;59(4):102769.