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

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**Author Contributions:**
Dr. Xinxin Huang contributed to the design of the study, analysis of the data and writing of the paper; Ms. Gina Pei Ling Gan, Mr. Kee Khiang Heng and Ms. Jing Jing Lee contributed to the laboratory testing, data collection and drafting of the paper; Ms. Susila Perumal, Ms. Rohani Salleh, Ms. Jessica Mei Ling Teo and Ms. Gaoge Xie contributed in performing the leukaphereses, data collection and writing of the paper; Dr. Esther Hian Li Chan, Dr. Aloysius Yew Leng Ho, Dr. William Ying Khee Hwang, Dr. Yeh Ching Linn, Dr. Yunxin Chen, Dr. Jeffrey Kim Siang Quek, Dr. Hein Than, Dr. Chandramouli Nagarajan contributed to the recruitment and assessment of the patients, guidance in the study, drafting and review of the paper; Dr. Francesca Lorraine Wei Inng Lim 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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The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Word Count:** 1495
Manufacturing chimeric antigen receptor T cells (CAR-T cell) requires collection of CD3+ lymphocytes through mononuclear cell (MNC) leukapheresis. MNC leukapheresis for autologous CAR-T cells manufacturing in patients with relapsed/refractory leukaemia and lymphoma who has 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 at al advocated processing a minimal of 12-15 litres of blood in order to harvest sufficient number of CD3+ lymphocytes for CAR-T cell manufacturing [2]. Patients often have sub-optimal performance status and physical reserve to tolerate such long leukaphereses. Lastly, patients with relapsed/refractory leukaemia and lymphoma usually have a small window period for successful leukapheresis where 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 pre-leukapheresis CD3+ count is the one common determining factor that has been repetitively mentioned. Pre-leukapheresis CD3+ count is difficult to be altered in patients with relapsed/refractory leukaemia and lymphoma. Hence, patients with lower pre-leukapheresis 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 pre-leukapheresis 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 pre-leukapheresis 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 cells manufacturing at the Singapore General Hospital (SGH), Department of Haematology from May 2020 to June 2021. The second set of data consists of 5 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 6 MNC leukaphereses for CAR-T manufacturing between 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 3 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: pre-leukapheresis CD3+ lymphocyte count (denoted as $C_{pre-leukapheresis}$, $10^9$ 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 to June 2021 were analysed to derive the mathematical model. Table 1 summarizes the basic demographic, pre-leukapheresis laboratory data, and collection data of the 12 leukaphereses.
Previous studies [5,6] described CD3+ lymphocytes collection efficiency (CE) as: 

\[ CE = \frac{T}{C_{pre-leukapheresis} * V_T} \]

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 5 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 \( C_{pre-leukapheresis} * V_T \) in above equation only had an R-square 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 removed from peripheral blood during leukapheresis. The rate of CD3+ lymphocyte collection gradually slows down as more blood 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 over-estimation of the CD3+ lymphocyte yield.

To account for the constant change in the “real-time” circulating CD3+ lymphocytes concentration, we made 2 assumptions: firstly the total amount of CD3+ lymphocytes circulating in the peripheral blood is not replenished from extravascular space during MNC leukapheresis; secondly the apheresis machine removes a fraction (\( \eta \)) 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 \( d(V_B * C_I) \) equals to the amount of CD3+ lymphocyte removed by the apheresis machine \( (C_2-C_I) * v * dt \). Therefore:

\[ d(V_B * C_I) / dt = (C_2-C_I) * v \]

By assumption 2, \( C_2-C_I = -\eta * C_I \). Solving the above differential
equation results in: \( C_1 = e^{\text{constant}} e^{(-\eta \cdot v \cdot t/V_B)} \). At \( t=0 \), \( C_1 \) equals the pre-leukapheresis CD3+ lymphocyte concentration, \( C_{\text{pre-leukapheresis}} \). Hence: \( C_1 = C_{\text{pre-leukapheresis}} e^{(-\eta \cdot v \cdot t/V_B)} \). \( v \cdot t \) equals the total volume of blood processed, \( V_T \). Hence, \( C_1 = C_{\text{pre-leukapheresis}} e^{(-\eta \cdot V_T/V_B)} \). The total CD3+ lymphocyte collected can therefore be expressed as: \( T = (C_{\text{pre-leukapheresis}} - C_1) V_B = C_{\text{pre-leukapheresis}} V_B (1 - e^{(-\eta \cdot V_T/V_B)}) \). This equation suggests that the maximum \( T \) from one leukapheresis is \( C_{\text{pre-leukapheresis}} V_B \), which is the total amount of CD3+ lymphocyte in the blood prior to leukapheresis. However, 10 out of the 12 leukaphereses performed at SGH between May 2020 to 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 5 leukaphereses at NUH, where 4 out of the 5 leukaphereses were able to obtain more CD3+ lymphocytes than what was 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 what we had assumed 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 dotted line), which results in an overestimation; whereas assuming an MNC leukapheresis is unable to yield more than the amount of CD3+ lymphocytes in the blood prior to leukapheresis (as shown in Figure 2 long dash line) underestimates the actual CD3+ lymphocyte yield. Therefore, we postulate that the relationship is likely a curve as shown in Figure 2 solid line: the speed of CD3+ lymphocyte collected will gradually slow down, but the total CD3+ lymphocyte collected should continue to increase as the leukapheresis continues. To describe the curve in solid line, we used a logarithm equation to approximate it, where \( a \) and \( b \) are constants unique to each apheresis.
centre: \( T = a \ln(C_{\text{pre-leukapheresis}} * V_T) + b \). 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 to June 2021, the equation for SGH was obtained: \( T = 3.588 \ln(C_{\text{pre-leukapheresis}} * V_T) - 2.006 \) (R-squared: 0.90). The residual standard error for this equation was 0.66.

The equation obtained was tested on the 6 subsequent MNC leukaphereses performed at SGH between June 2021 to April 2022 and it had an R-squared value of 0.91. This showed that the equation obtained from previous data was still applicable for subsequent leukaphereses in the same centre. 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-squared value of 0.97.

Previous published literatures have described variables with predictive value for the CD3+ yield, including CD3+ count and haematocrit in one study [3] and CD3+ count, haemoglobin level, and platelet count in the other [4]. Despite some differences, CD3+ count is consistently the most important variable that impact the final CD3+ yield. What the current model adds to the existing ones is that the current model is a generalisable model. It requires each centre 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 pre-leukapheresis CD3+ lymphocyte count and the volume of blood processed. The equation \( T = a \ln(C_{\text{pre-leukapheresis}} * V_T) + b \) is a generalisable equation 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.
References

Table 1. Summary of basic demographic, pre-leukapheresis laboratory data, and collection data for the 12 leukaphereses at SGH between May 2020 to June 2021.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range (min – max)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (female)</td>
<td>10 (91%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (year)</td>
<td>54</td>
<td>19 – 73</td>
<td>16</td>
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<tr>
<td>Height (cm)</td>
<td>160.7</td>
<td>148 – 179</td>
<td>8.3</td>
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<tr>
<td>Weight (kg)</td>
<td>56.6</td>
<td>40 – 85.1</td>
<td>12.5</td>
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<tr>
<td>BMI</td>
<td>22.0</td>
<td>16.3 – 30.0</td>
<td>4.0</td>
</tr>
<tr>
<td><strong>Pre-leukapheresis laboratory data</strong></td>
<td></td>
<td></td>
<td></td>
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<td>Hemoglobin level (g/dL)</td>
<td>10.28</td>
<td>7.8 – 12.5</td>
<td>1.41</td>
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<td>Hematocrit (%)</td>
<td>30.63</td>
<td>24.3 – 35.2</td>
<td>3.60</td>
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<td>Lymphocyte count (10^9 cells/L)</td>
<td>0.71</td>
<td>0.23 – 1.67</td>
<td>0.44</td>
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<tr>
<td>Pre-pheresis CD3+ lymphocyte count (10^9 cells/L)</td>
<td>0.57</td>
<td>0.20 – 1.49</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Leukapheresis data</strong></td>
<td></td>
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<td></td>
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<td>Total body blood volume (L)</td>
<td>3.57</td>
<td>2.80 – 5.45</td>
<td>0.68</td>
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<tr>
<td>Total blood volume processed (L)</td>
<td>10.19</td>
<td>5.52 – 16.78</td>
<td>2.67</td>
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<tr>
<td>Total collection time (minute)</td>
<td>319</td>
<td>117 – 410</td>
<td>59</td>
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<tr>
<td>Total mononuclear cell collected (10^9 cells)</td>
<td>12.25</td>
<td>5.75 – 19.65</td>
<td>4.90</td>
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<tr>
<td>Total CD3+ lymphocyte collected (10^9 cells)</td>
<td>3.57</td>
<td>0.67 – 7.66</td>
<td>1.97</td>
</tr>
</tbody>
</table>

*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.

Legend to Figures

Figure 1. A simplified illustration of the leukapheresis process. \( C_1 \) is the concentration of CD3+ lymphocyte in the patient’s peripheral blood at any time of leukapheresis; \( C_2 \) is the concentration of CD3+ lymphocyte in the blood leaving the apheresis machine returning to the patient; \( V_B \) is the total body blood volume; \( v \) is apheresis machine blood flow rate; \( T \) is the amount of CD3+ lymphocyte 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+ lymphocyte 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 to be a curve shown in the solid line.