WHITE PAPER

A Comprehensive Analysis of Fresh Apheresis Collections: Conclusions and Best Practices

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EXPERIENCE MATTERS

Successful commercialization of cellular therapies is critically dependent on providing a consistently high-quality product. So, what defines a superior cell therapy product and how is it accomplished? At HemaCare, we understand that a critical component of success lies in starting with apheresis material of the highest achievable quality. Creating reliable donor networks and optimizing apheresis product yield and purity are two of the most important factors that will determine the quality and efficacy of the final therapeutic product.

Cellular therapy is exceptional in that the starting material is inherently variable because it originates with human donors; each with a distinctive physiology, lifestyle, and medical history. This variability makes creating a medical treatment with consistent quality and efficacy challenging. At the same time, the people on the receiving end of these innovative new therapies stand to benefit from a treatment tailored to their physiology. Designing a therapy that only caters to the "average" patient is not practical in a world filled with a diverse population. Rather, it is ultimately more practical to strive for optimizing and standardizing as many supporting factors as possible during starting material production, so that variability is derived from donors alone. Our goal at HemaCare is to produce a consistently high-quality product, while also sharing our deep understanding of the factors contributing to natural population variance in white blood cell numbers and cell type distribution.

Experience with donor center operations is a vitally important part of creating consistent, high-quality starting material. Even in an era of innovative biomedical strategies and automated cell handling and processing methods, apheresis remains more of an art than a science. Apheresis nurses and staff need to become trained in a unique combination of skill sets. HemaCare has been working with apheresis donors for over 40 years; the donor center relies on an expert staff with over 250 combined years of clinical experience and over 100 combined years of donor center experience. Together, they have performed over 300,000 successful apheresis collections. That level of proficiency simply can't be duplicated.

Along with clinical experience, apheresis staff requires training with the instrumentation itself, which may vary from one clinic to the next or one type of cell collection procedure to the next. Being familiar with the details of various collection methods are just as important; for example, how to adjust flow rates and collection volumes to accommodate each unique patient, which mobilization techniques are best suited to individual donors and clients, and which collection methods are best suited for a particular target cell. Optimizing cell collection efficiency is the key to maximizing the yield and purity of each apheresis run.

HemaCare scientists are experienced with optimal cell handling techniques, including cell preservation and storage parameters, and transport logistics. Staff is highly knowledgeable about the various data collection systems used to track donor biometrics and link collection methodologies to apheresis unit yield and quality. A well organized and detailed apheresis donor database can help both collection centers and their clients quickly search for the donors who will best meet the needs of a given project.

APHERESIS DONOR NETWORK

HemaCare has established the largest reliable and recallable donor database in the industry. That takes a lot of dedicated effort and sustaining that database is HemaCare's ongoing mission. HemaCare actively recruits

healthy donors. An apheresis donor database must be continually renewed through active outreach to both new and long-term donors. This effort includes referrals from current donors, social media and digital outreach, and educational outreach. Recruitment efforts are carried out monthly, with an emphasis on establishing credibility through consistent positive donor interactions built on genuine care.

Donors are informed in detail about what the donation procedure entails, including any possible risks or adverse reactions. They are also kept informed about how their donation will be used, which research programs will benefit, and how vital such donations are to support medical research in general.

HemaCare's apheresis center currently performs thousands of apheresis procedures per year. A significant number of these are collected from repeat donors.

Access to reliable, recallable donors is particularly important to manufacturers who know they will need to duplicate project-specific physiological or demographic traits that may otherwise be difficult to source in a timely manner. For example, developers may ask to add a certain percentage of donors to their project who are willing to commit to additional donations later during project development. Donor planning minimizes the risk of delays due to inadequate starting material. The most valuable characteristics cell therapy developers look for in donors are those which meet specific project criteria, are reliably responsive to outreach, and have good WBC yields.

To maximize the value of their donor network, HemaCare recruits donors across a wide range of demographics and selection criteria. A nurturing environment and a strong commitment to donor safety is essential to ongoing recruitment efforts and to encouraging repeat donations. All cellular products are required to be collected from consented donors following IRB-approved protocols. To protect donors as well as clients, HemaCare implements a more extensive and more thorough than average screening panel for infectious disease prior to leukopak collection, including, at minimum, screening for HIV, Hepatitis B, and Hepatitis C. Complete donor histories are recorded to aid with the client selection process. Blood type is verified, along with biometric data such as age, gender, ethnicity, BMI, medical history, and when requested, HLA-typing. In addition, data on lifestyle characteristics such as smoking, drinking, and other stress factors are recorded. All qualification and screening processes are monitored as part of the rigorous quality systems oversight HemaCare has in place.

STARTING MATERIAL VARIABILITY

Cell therapy developers should keep in mind that variability is an inherent trait of living cells and tissues. While most people anticipate variability in disease-state samples, where physiological impacts are expected, fewer people fully realize how much variability exists even amongst healthy donor samples.

All donors are unique. Differences in age, size, gender, and other factors will affect apheresis results (Nishitani 2013, Nah 2017, Jamshidi 2015). Donor lifestyle habits can also impact collection. Regardless of coaching or advising donors on healthy lifestyle choices, donor behavior and habits are unpredictable. Diet, drinking, smoking, stress levels, and sleeping habits have all been determined to impact WBC numbers and cell or phenotype distribution (Ackerman 2012, Nishitani 2013, Riley 2015). Apheresis centers can make predictions based on experience, but there will always be donor-to-donor and collection-to-collection variability that can't be fully anticipated.

To gain a better understanding of the amount of variability apheresis centers encounter on a regular basis, HemaCare recently carried out an in-depth analysis of a representative sample of their fresh leukopak collections drawn from healthy donors (Fig.1).

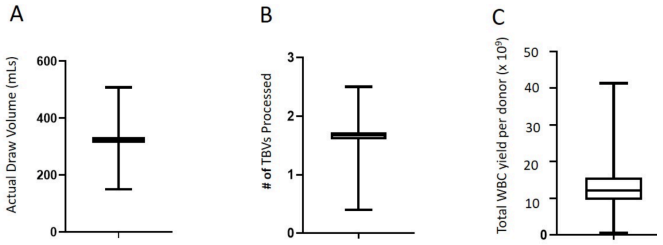

Figure 1: Analysis of HemaCare's Apheresis Collection
Cohort showing descriptive statistics for 2490 collections from healthy donors. Bars indicate standard range of values with error bars.

Apheresis draw volume depends on a number of donor-dependent variables; primarily it is calculated as a percentage of their total blood volume, but factors such as medical history and whether donors experience any adverse events will also impact the collected volume. Researchers examined normal distribution ranges in regard to apheresis unit yield and composition. In a cohort of 2490 procedures, our apheresis centers collected draw volumes varying between 150 mLs-508 mLs, with a mean of 318 mLs drawn. These collected draw volumes result from processing a mean of 1.64 total blood volumes (TBV) per donor. Total WBC yields varied between 0.5-40.1 billion (x 10^9) cells/donor, with a mean yield of 12.7 x 10^9 cells/donor.

Additional analysis of white blood cell yield frequency was performed (Fig. 2). WBC counts are used by physicians as an indicator of potential disease presence. Abnormally high WBC counts, for example, can indicate infection, inflammation, or certain types of cancer, while abnormally low WBC count can indicate viral infections, cancer, or autoimmune disorders. Therefore, WBC yields outside of the normal range may indicate the donor is

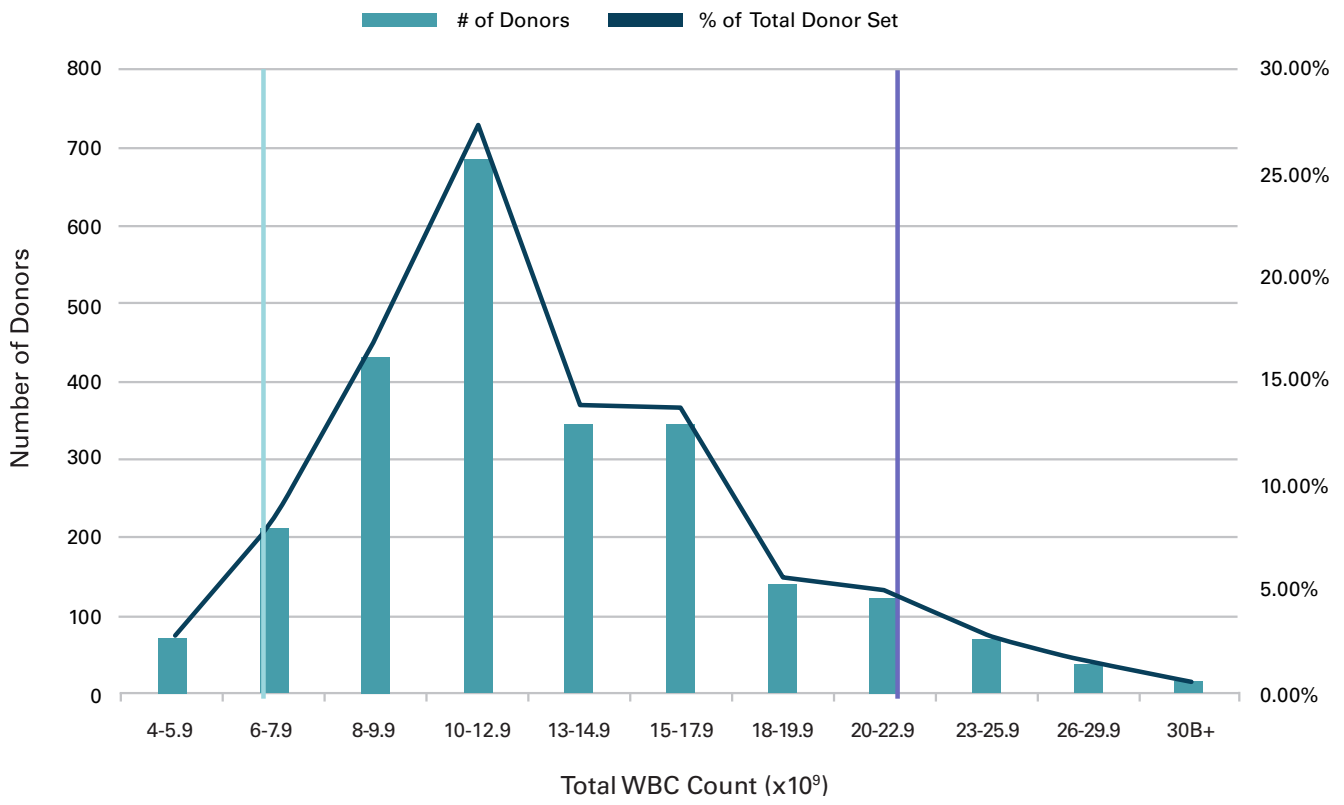

Figure 2: WBC Yield vs Total Number of Donors
Analysis of white blood cell yield and frequency in 2490 apheresis donors with mean yield being 12.7 x 10^9 cells/donor and 5th (light blue line) and 95th (purple line) percentiles of 6.4 and 22.9 x 10^9 (cells/donor).

impacted by illness. Higher WBC yields *within* the normal range, on the other hand, generally indicate a higher number of therapeutic target cells.

Despite wide variance, the majority of data points cluster near the mean. Because healthy donors have a wide distribution in WBC cell counts, exercise caution when selecting for a specific range.

As part of HemaCare's study on the 2490 leukopak collections, the scientific team also assessed the WBC count for varying blood volumes of apheresis units processed (Fig. 3). HemaCare processes roughly 1.5-1.8 total blood volumes (TBV) in the majority of donations, which is equivalent to 8-9 liters of blood in the majority of donations. There is no significant variance in WBC counts in apheresis collections varying between 1.0 to 2.0 processed TBVs. That WBC recovery is relatively consistent at nearly all blood volume ratios suggests that the TBV processed does not play a major role in total WBC yield per apheresis unit. Importantly, increasing TBVs processed beyond an ideal point, especially using suboptimal collection parameters, may lead to an increase in undesirable cellular subsets due to increased physical stress on the donor.

While these recent analyses demonstrate the standard variance one can expect within a healthy donor population, cell therapy developers are naturally focused not merely on overall cell yields, but more particularly on the number of target cells of interest contained within an apheresis unit. As such, HemaCare analyzed a smaller subset of 70 leukopaks for which flow cytometry had been performed to assess the frequency/yield of key target cell populations. Results of the average percentage of major WBC subpopulations are shown (Fig. 4). Within the examined 70 cohort cell collection, the largest percentage of cells, 47.2%, were identified as T cells, while monocytes comprised 19.5%, NK cells 13.6%, and B cells 9.2%, with an additional 10.5% of cells not clearly identified.

In order to elucidate and examine in depth the inherent variability present in donor populations, the analysis was expanded to scrutinize the population frequency of various T cell subtypes. Results of the analysis for CD3+, CD4+, and CD8+ T cell populations are shown (Fig. 5).

As with total yields, percentages of specific cell subpopulations vary from donor to donor. In this 70-cohort subset of fresh leukopak collections, for instance, the percentage of CD3+ cells ranges from 24.4-68.3%, with an average of 47.2%, as stated above (Fig. 5A). Of this CD3+ population, CD4+ versus CD8+ cell frequency is 31.6% and 14.6%, respectively. Therefore, in an average leukopak containing 12.7 x 10^9 WBCs, the observed percentages would translate to a mean CD3+

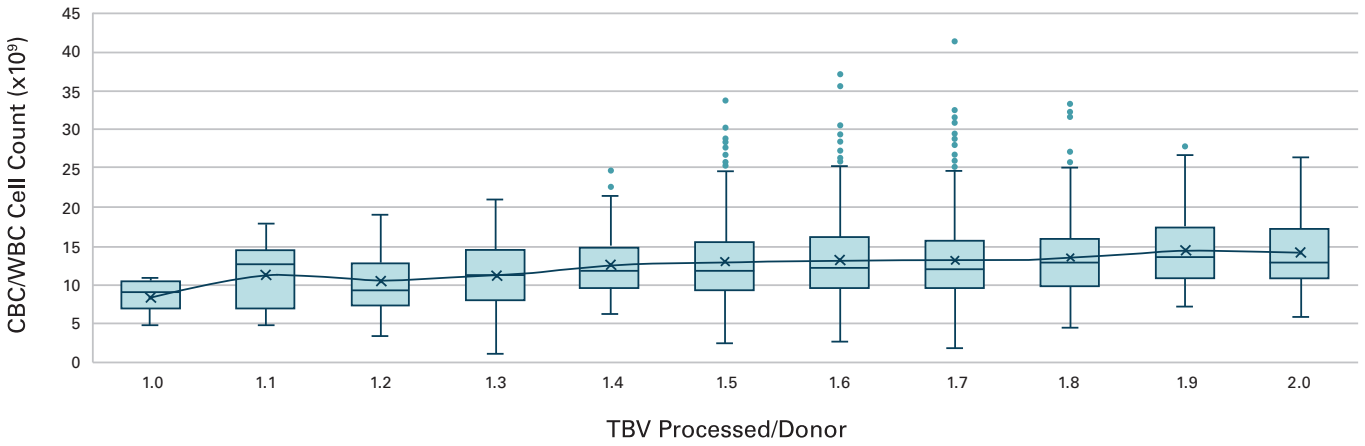

Figure 3. CBC/WBC Count vs Total Blood Volume (TBV) Processed

Mean (X) and median (line within bar) indicate the WBC counts observed for each TBV processed. Bars indicate standard range of values with error bars and major outliers present as points outside of the error bars.

cell count equaling roughly 6.0 x 10^9 cells and anticipated average CD4+ and CD8+ cell counts around 4.0 x 10^9 and 2.0 x 10^9 cells, respectively (Fig. 5B). Although the anticipated mean cell numbers extrapolated in Fig. 5B do provide a great estimate and certainly hold true when a population is analyzed, they are only averages and must be seen through this lens. In the 70-donor cohort analyzed, 48, 49, and 65% of donors have greater than the anticipated average cell yield of 6.0 x 10^9 CD3+ cells, 4.0 x 10^9 CD4+ cells, and 2.0 x 10^9 CD8+ cells, respectively (Fig. 5C). It should be noted, however, that actual cell numbers ranged from 1.8-19.4 x 10^9 CD3+ cells, 0.93-13.1 x 10^9 CD4+ cells, and 0.49-8.4 x 10^9 CD8+ cells, thus demonstrating again the level of variability that is observed between donors.

Other white blood cell types display a similar donor-to-donor variability (Fig. 6). The percentage of NK cells and B cells that make up the total WBC population in the same 70-donor cohort varies from 6.2-22.6% per donor and from 3.1-18.6% per donor, respectively. Both of these cell types are commonly used as starting material for cell and gene therapy development.

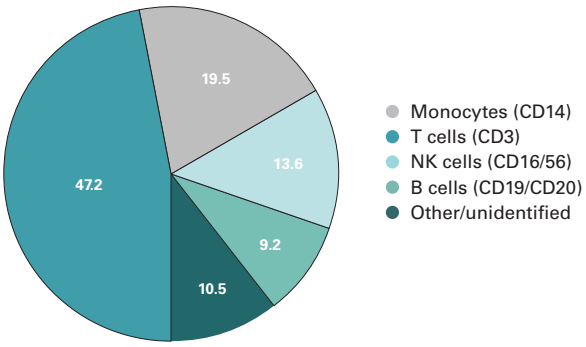

Figure 4. WBC Subpopulations (percent total)

Mean percentages of individual WBC types as measured by flow cytometry.

The take-home point of this preliminary WBC subpopulation analysis is that there will always be donor-to-donor variability. This is ultimately beneficial to the cell therapy industry, because patient populations are diverse. Manufacturers want a product that has efficacy for the highest number of patients possible.

Of course, there are specific instances in which limiting donor variability is essential, for example, during development of allogeneic therapies, to demonstrate process consistency, or during long-term clinical trials. In these cases, access to reliable, recallable donors is beneficial. Nonetheless, the most important thing a starting material supplier can do is not to eliminate donor-related variability, but rather to make sure handling and collection practices are optimized so that variability doesn't impact quality.

WBC collection volume and yields are naturally limited by donor safety considerations. While it makes sense to strive for a higher yield, caution should be exercised. Donors with a lower BMI will naturally have a lower cell collection volume and contriving to stretch that volume may simply lead to a higher percentage of contaminating cell types or a larger percentage of cells damaged by high flow rates (Radley, 2018). Even before considering specific donor demographics, it is evident that there is a wide variation in apheresis results even amongst healthy donors. Optimal separation of blood components, while it may yield a lower volume leukapheresis product, can help guarantee higher target cell purity from the start and result in lower target cell loss due to a minimized need for further separation steps (Golab 2016, Anyanwu 2018). Superior protocols and processes guarantee higher product purity and better yields of those cells that contribute the most to downstream efficacy. Optimal collection and sample handling methodologies go a long way toward preserving consistently high-value apheresis collections.

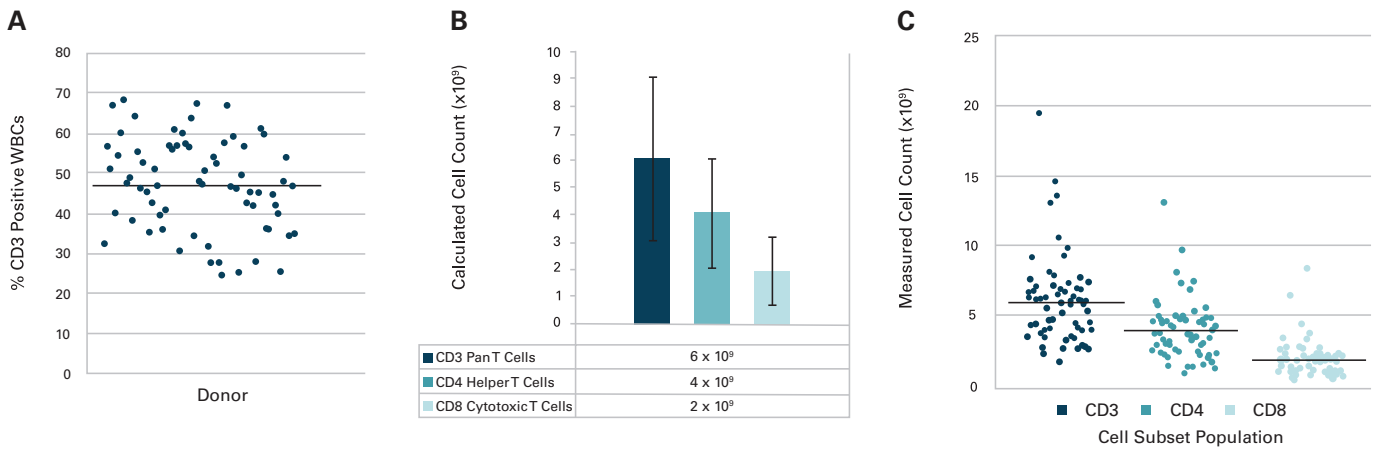

Figure 5. WBC Subset Population Ratios Correlate with Anticipated Average Calculations, Despite Donor Variability
CD3 cells average 47.2% of the total WBC population collected (Panel A). In an average leukopak of 12.7 x 10^9 cells, this would correlate to anticipated average CD3 counts equaling roughly 6 x 10^9 cells, and CD4 and CD8 counts around 4 x 10^9 and 2 x 10^9 cells, respectively (Panel B). Cell subset populations measured by flow cytometry correlate well with the anticipated average calculation (Panel C). The black horizontal line shows mean value.

SUMMARY

Over the last few years, the healthcare industry has witnessed significant growth in the number of cell and gene therapies being developed. As an increasing number of these therapies mature towards commercialization, the need for high-quality starting material will grow proportionally.

Sourcing material for the development of a cellular therapeutic is one of the most important decisions a manufacturer can make. To make informed decisions when choosing a supply partner or partners, manufacturers should look for an extensive, diverse network of reliable, recallable, and highly-characterized donors. Apheresis and cell collection center staff should be knowledgeable about what characteristics to seek in a donor and how demographic and lifestyle traits might impact apheresis yield and quality.

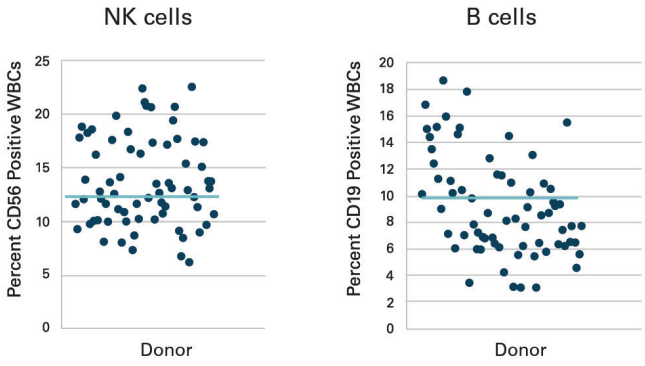

Figure 6. Percentage of the Total WBC Population/Donor
Shown for NK cells and B cells. Cell subset populations are measured by flow cytometry. The light blue horizontal line shows mean value.

An experienced cell therapy starting material provider is familiar with the wide range of natural variation present in apheresis material from both healthy and disease-state donors. By tracking donor demographics and analyzing the number and type of cells present in each cell collection, a greater understanding is gained concerning the impact of distinct donor-dependent factors on target cell viability and functionality. Total collected volume and total WBC yield are good preliminary indicators of apheresis unit quality, but one must also take into account contributing factors such as donor age and BMI, cell type distribution profiles, and total nucleated cell counts.

Training and equipment should be standardized as much as possible between collection centers, and collection center staff should be proficient not only with donor selection and recruitment, but also with the more technical aspects of apheresis, such as operating apheresis instruments, choosing the best reagents and supplies, optimizing collection efficiency, and managing data collection systems.

HemaCare understands the impact of starting material variability on cell and gene therapy products and collaborates with clients and partners to develop unified guidelines on topics ranging from donor center operations, to donor qualification and characterization, to quality control systems. When it comes to performing an actual apheresis procedure, experience goes a long way, since healthcare staff need to be experts on a wide variety of factors. Medical knowledge, donor management, and donor safety are paramount. Furthermore, expert knowledge of apheresis equipment, cell collection methods, and sample handling are contributing factors that help collection center staff balance apheresis yield

with purity, thereby maximizing collection efficiency and maintaining optimal cell viability and functional capacity.

In conclusion, variability will always be part of the cell therapy industry. For the first time, drug developers are having to deal with the fact that they are not starting with uniform raw materials. As starting material providers, we can use best practices and standardization to promote consistent quality and efficacy. Training, education, and experience can all counteract intrinsic variability, and through careful donor selection and stratification, we can bring increased uniformity to starting material cohorts for individual research projects. A better understanding of the factors impacting starting material sourcing and quality will benefit the industry as a whole.

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