Lymphoma Epidemiology of Outcomes

Newsletter - Year 8 **2023**

Learn how your LEO contributions help improve lymphoma treatment.

What's Inside:

- An interview with LEO Investigator Dr. Anne Novak, from Mayo Clinic Division of Hematology
- Travel through the life of a sample after it is drawn
- The real-world impact of LEO research
- What to expect when you receive an electronic survey

7 years of research

8 participating centers

Over **70** publicationS

104 LEO personnel

9,340 participants

150,685 blood samples

"From Draw...

...To Discovery"

Flowchart on the pathway of the blood

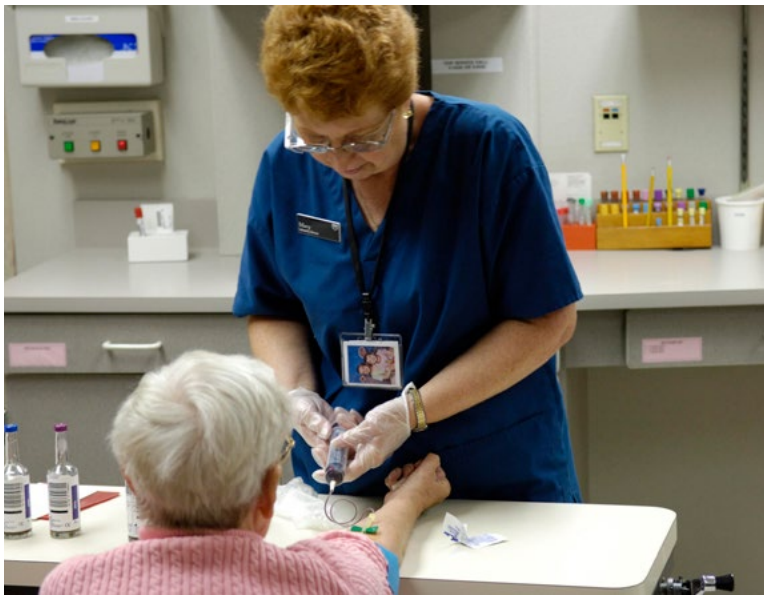
The LEO study consists of 8 medical centers and academic institutions across the country.

Because of your participation, we are able to collect data and samples that will help researchers better understand and treat lymphoma.

But once these samples are collected, what happens to them and where do they go?

With this flowchart, you can follow along on the journey that your blood donation makes and how it eventually ends up in the hands of researchers. Your donation is used for important studies that helps future patients just like you.

1. Venipuncture - blood draw



Blood is first drawn from the participants using **venipuncture** techniques. It is ported into vacutainers, which are the tubes that hold blood.

These tubes may have additional agents that change the composition of the blood in the tube. These agents allow clinicians and scientists to run different tests on the blood.

In LEO, two types of vacutainers are used:

- The red-stoppered “No-adds” have no additional chemicals, and the blood is able to clot in the tube.
- The purple- top “EDTA” tubes remove calcium from the blood, which prevents it from clotting.

For most LEO participants, study blood is drawn at the same time as clinical labs to prevent additional needle sticks.

2. Blood is transported to the lab

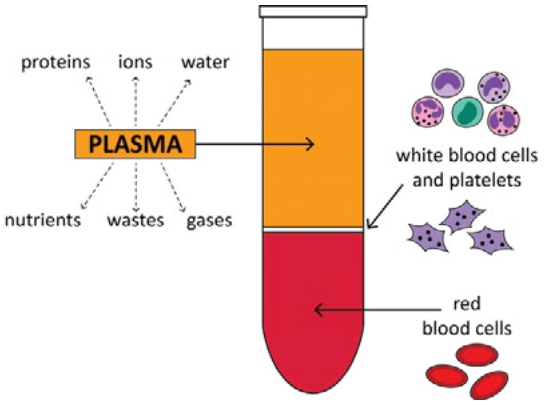
After being drawn, the blood is transported to our research laboratory where it is allowed to clot before being processed. The No-add tubes are allowed to rest for 30-60 minutes so that they can clot before being processed. In comparison, the EDTA tubes go straight to processing.

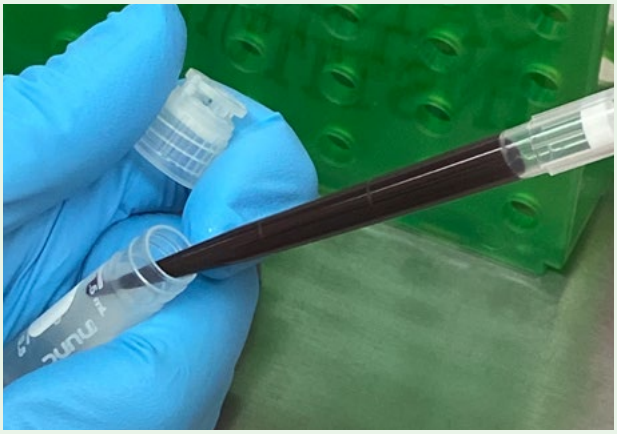
3. Preparing the bloods

The tubes are put in a device called a centrifuge that spins at very high speeds, separating the blood into its components by density. From there, technicians are able to isolate the different testable parts of the blood such as serum, plasma, white, and red blood cells, which are of use to researchers.

Plasma and serum are both the liquid portion of blood. However plasma, red, and white blood cells can only be obtained from un-clotted blood, which is why the EDTA tube is used. Serum is the liquid left over after blood has clotted, and so it is extracted from the No-add tube.

Technicians pipette the blood components into individual smaller vials to maximize the number of samples created from one tube of blood. These smaller (cryo)vials are put into -80° Celsius (-112° Fahrenheit) freezers and stored until they are shipped on dry ice to Mayo Clinic in Rochester, Minnesota.





4. Sending the samples to Mayo Clinic

When research sites ship the collected blood samples to Mayo Clinic, they use dry ice and styrofoam containers to keep the samples cold and safe in transit.

5. Stored at Mayo Clinic Biospecimen Accessioning and Processing (BAP) Core at -80° Celsius

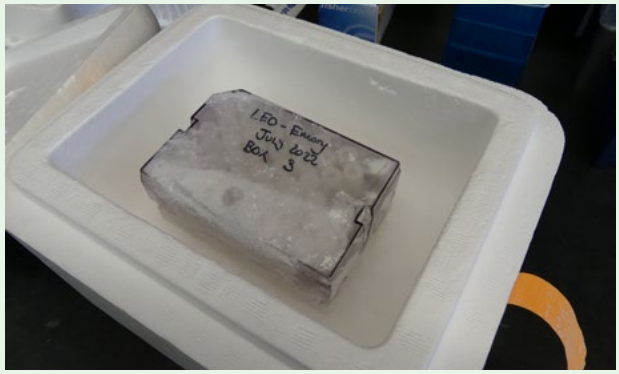
Once the samples arrive at Mayo Clinic, they are directly put into a -80° Celsius freezer. Sometimes, depending on the type of sample or how long researchers are going to keep a sample, it is put into a tank of liquid nitrogen, which is kept at -196° Celsius.

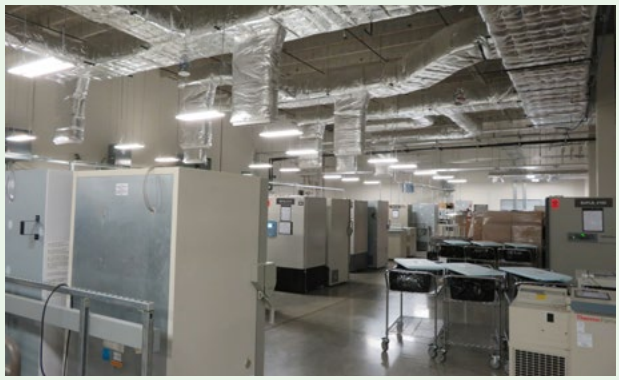
It's important for these samples to be kept extremely cold so the molecules in the blood do not degrade over time.

6. Extracted samples are thawed

Once the samples are thawed, they are ready to be used and tested for research studies. On the next page you can follow along with a real-life example of how samples were used for a research study.

The BAP lab is a one-of-a-kind storage and processing facility custom built for Mayo Clinic in Rochester, MN. The BAP lab can hold up to 35 million research tubes and is robotically controlled!





Researchers are constantly thinking about how best to keep and use these extremely valuable samples. Before samples are used, researchers will discuss and approve what will be used for a study and what will be saved for a future study.

These are some of the first questions asked before using a sample:

"After an extraction, will the samples be exhausted or still usable for other studies?"

"How many times can this sample be frozen & unfrozen before it affects the quality of the sample?"

The Story of a Sample

Every study starts with a question and it leads to a written plan called a protocol. After the plan is finalized, funded, and approved, the real story of the sample begins. Below is a real-life example of how a hypothesis turned into a study, and how it eventually lead researchers to discover important new information on lymphoma outcomes and led to a clinical trial.

Vitamin D Insufficiency and Prognosis in Non-Hodgkin's Lymphoma Study

1. Sample is selected for the study:

Every study begins with generating a list of eligible participants. A participant could be eligible for a study based on diagnosis, treatment type, and availability of blood or tissue samples. For example, this study looked at all Non-Hodgkin's Lymphoma diagnosed participants with serum samples. One serum sample was retrieved from the freezer for each eligible participant.

2. Laboratory tests are run:

The sample is sent to the laboratory for testing. This study tested serum samples for Vitamin D levels.

3. Test that data and clinical variables are aligned:

The laboratory sends the Vitamin D levels to the statisticians, who align the results with the clinical information for each participant.

4. Statistical Analysis:

A. The statistician checks for unknown relationships and creates the statistical analysis plan. In this study, the statisticians examined the correlation between the Vitamin D levels and each clinical variable such as age, gender, diagnosis, or resident state at diagnosis. These results are reported in a table (See Table 1).

B. Next the statistician performs survival analysis. The goal of this analysis is to determine if a participant's Vitamin D level is related to how long a participant lived or lived without having their cancer getting worse.

5. Conclusions and Publication:

The statistical analysis showed that having sufficient Vitamin D was associated with better overall and event-free survival of patients with Non-Hodgkin's Lymphoma. The findings of this study were persuasive enough to start a clinical trial to validate Vitamin D's role in NHL progression and survival.

Vitamin D Insufficiency and Prognosis in Non-Hodgkin's Lymphoma

Drake, M.T., et al., Journal of Clinical Oncology 2010 28:27, 4191-4198

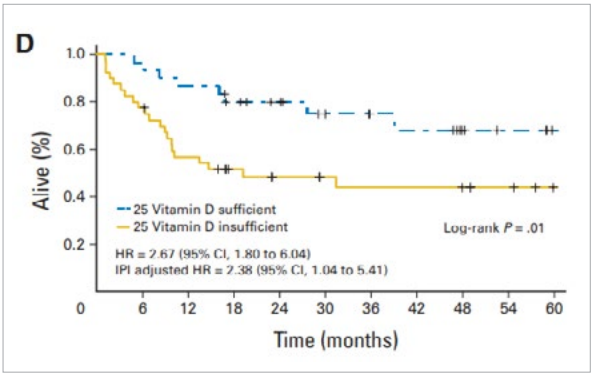
Table 1.

Table 1. Demographic and Clinical Correlates of Vitamin D Levels and Prevalence of 25-Hydroxyvitamin D Insufficiency

Covariate	No.	25-Hydroxyvitamin D Levels (ng/mL) Mean	SD	25-Hydroxyvitamin D Insufficient No.	%	P*
Timing of serum draw, < 120 days of diagnosis						
Pretreatment	649	27.4	10.4	265	40.8	.002
During or post-treatment	334	24.8	11.1	171	51.4	
Sex						
Male	540	26.3	10.2	242	44.8	.75
Female	443	26.7	11.3	194	43.8	
Age, years						
≤ 60	458	26.4	10.7	204	44.5	.91
61+	525	26.5	10.8	232	44.2	
Residence at diagnosis						
MN, IA, IL, WI, ND, SD	874	26.2	10.6	399	45.7	.02
Outside six state region	109	29.0	11.3	37	33.9	
Month of diagnosis						
March-May	212	25.0	10.1	110	51.9	.02
June-August	249	28.3	10.1	94	37.8	
September-November	265	27.0	11.5	114	43.0	
December-February	257	25.5	10.8	118	45.9	
Performance status						
0 or 1	845	27.7	10.3	336	39.8	< .001
> 1	137	18.8	10.3	100	73.0	
Subtype†						
DLBCL	370	24.7	10.7	192	51.9	.02
TCL	70	23.2	11.8	40	57.1	
MCL	71	27.0	9.1	26	36.6	
FL	285	28.2	10.3	110	38.6	
Post-FL	109	28.8	11.0	41	37.6	
All other	78	28.1	10.5	27	34.6	

This table shows the number of participants and their Vitamin D level in comparison to a control group. It also shows a p-value, which tells readers if there is a meaningful difference in Vitamin D levels between lymphoma patients and the control group. A p-value <0.001 tells the researcher that the difference is not coincidental, meaning that there could be a medical reason the Vitamin D levels are low. It does not tell the researcher why.

Figure 2.



This graph is called a Kaplan-Meyer curve and it demonstrates the difference in survivorship between Vitamin D sufficient and insufficient lymphoma patients. The Hazard Ratio (HR) is shown in the bottom left corner and indicates that a participant who has DLBCL and is Vitamin D insufficient is 2.67 times more likely to pass away than a participant who has DLBCL and is Vitamin D sufficient.

Interview with Dr. Anne Novak, Ph.D., Associate Professor of Medicine at Mayo Clinic in Rochester, MN

"What is your oncology research background?"

Cancer is fundamentally a genetic disease, and my research program uses genetic data to guide our ability to identify newly diagnosed lymphoma patients at risk of early clinical failure and an aggressive disease course. These same data are also used to better understand the underlying biology of aggressive lymphomas in hopes to identify novel targets for therapeutic intervention. Participant samples collected as part of LEO are critical for the success of these studies. The data collected are carefully maintained and may be shared with other researchers in LEO to help support our lymphoma research community and all patients.

"Why are tissue samples essential for this study?"

Clinically obtained tumor tissue biopsies and research blood samples are the most common sample types used in genetic studies. These sample types provide information on a patient's normal healthy tissue and the tumor, which allows us to understand how they are different. The genetic data collected from the samples is not shared outside of the research community and cannot be linked back to a participant. These types of studies provide powerful information to researchers to help understand how lymphoma arises, how it progresses, and how it may respond to therapy. Recent studies highlight how these data can also be used for individualized therapy and precision medicine approaches.

"What are you most excited about for the future of LEO?"

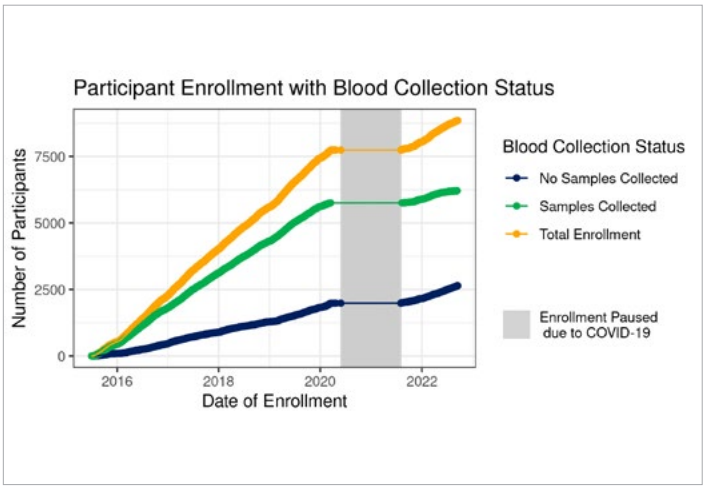
LEO is an amazing resource due to the large numbers of patient derived samples with matched clinical information. However, one of the most impactful contributions that LEO can make is through its collection and study of rare lymphomas. When a research community such as LEO collaborates, it allows for us to study diseases that would take years for any individual group to collect information on.

Another area of high impact is through its collection of serial samples from the same patient over time. This allows us to gain insight into how lymphoma patients respond to therapy, how they may be cured, or how disease may relapse over time.

"Most important take-away for a participant on this study."

A key step in finding a cure for lymphoma is to study it, and for that to happen we need samples as well as clinical and outcome data from patients. As a laboratory-based basic science researcher, my goal is to work with clinicians to understand lymphoma, to identify patterns in data, to discover new biology and discover new treatments. Every sample we use derives from a participant with lymphoma, a person in search of a cure, and the most important take-away is that we use these data to strive for the same goals.

Figure 3.



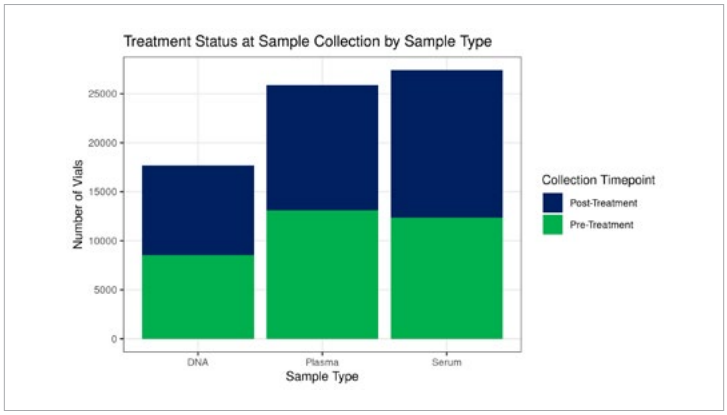
Not every LEO participant agrees to, or is physically able to contribute a blood sample. This plot shows the total number of LEO participants in yellow, the subset of those patients who did contribute blood in green, and the subset of those patients who did not contribute blood in blue. This plot indicates that roughly 70% of LEO participants do contribute blood to the LEO study.

How is LEO making a real-world impact?

Dr. Arushi Khurana, M.B.B.S. is a hematologist-oncologist at Mayo Clinic in Rochester, MN and a LEO investigator. Dr. Khurana was selected for the American Society of Hematology (ASH) Press rogram for her recent abstract. This prestigious award allowed Dr. Khurana the opportunity to share her findings on a broader platform at the 2022 ASH annual meeting.

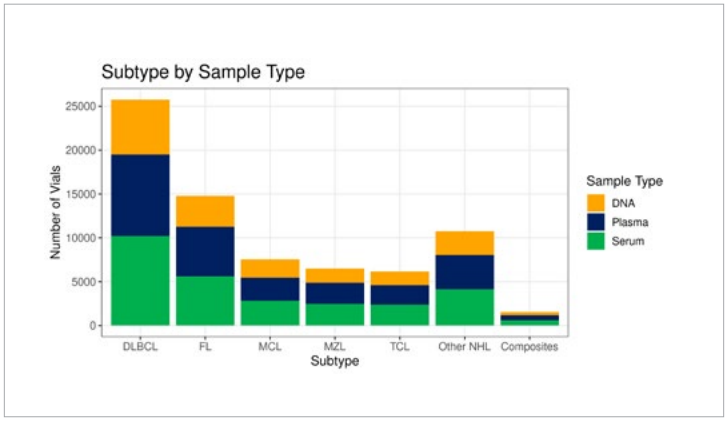
Dr. Khurana has brought light to an on-going problem and has helped influence clinical trial developers to re-think previous criteria to better serve all cancer patients. This study is one example of the many research questions the LEO cohort can address thanks to your participation. Please see a summary of this important abstract below.

Figure 4.



The timing of when a patient contributes blood to the LEO Cohort during their lymphoma journey can matter to an investigator's research question. For example, an investigator looking to find a new way to diagnose lymphoma earlier would need to use blood samples that were collected from lymphoma patients before their treatment started. However, an investigator looking for hereditary lymphoma genes could use blood samples collected at any time because no treatment can change those genes.

Figure 5.



This image includes bars for each lymphoma diagnosis subtype. The height of the bars indicates the total number of blood based samples collected from LEO patients.

Evaluating the Impact of Lab-Based Eligibility Criteria By Race/Ethnicity in Frontline Clinical Trials for Diffuse Large B-Cell Lymphoma (DLBCL): A LEO Cohort Analysis

In order to be considered for certain clinical trials, you must meet certain eligibility criteria. This could include your age, diagnosis, previous treatment, or even lab-based values such as hemoglobin level. In a previous analysis by our group, it was found that that almost a quarter of patients diagnosed with DLBCL were unable to join frontline clinical trials due to their lab-based values not meeting the eligibility requirements. It was also found that those same ineligible patients suffered worse health outcomes and had an increased chance of dying from their lymphoma in comparison to their peers who were able to participate on clinical trials.

In this new analysis using LEO cohort data, the objective was to find out whether race/ethnicity affected a DLBCL patient's ability to join frontline clinical trials. We found that Hispanics and non-whites were diagnosed at a much younger age than their white peers, their hemoglobin levels were significantly lower, and oftentimes their treatment regimens differed. Similarly to the previous study's findings, 9-26% of LEO participants with DLBCL were unable to join many frontline clinical trials because they did not meet the lab-based eligibility criteria.

In conclusion, certain and often strict eligibility criteria can leave behind a large percentage of DLBCL patients who would otherwise be willing to join clinical trials. This seems to affect Hispanic and non-white patients at a higher rate, as they are more likely to not meet the eligibility requirements for DLBCL clinical trials.

Lymphoma Epidemiology of Outcomes

What to expect when you receive a LEO questionnaire:

In order for us to continue this important research, we depend on our participants to answer our standardized research surveys. We ask all our participants to fill out these surveys throughout their lymphoma journey including throughout their remission. Depending on your selected contact preferences, you will receive the following communication from the LEO cohort via mail or email. If you receive this communication through email, it will come from:

LEO (Lymphoma Epidemiology of Outcomes) Follow-up Survey [mailto:noreply@qemailserver.com]

From: Lymphoma Epidemiology of Outcomes (LEO) Research Study: Follow-up Survey [mailto:noreply@qemailserver.com]
Sent: Tuesday, August 25, 2020 12:04 PM
To: Farmer, Sara A.
Subject: [EXTERNAL] Lymphoma Epidemiology of Outcomes (LEO) Research Study Follow-up Survey Request

REMINDER

Dear Participant,

Thank you for your continued dedication to The Lymphoma Epidemiology of Outcomes (LEO) study. Due to the generosity of individuals like you, the experiences and outcomes of people with lymphoma are better understood than they were a decade ago. Research continues on the information collected to date and is frequently updated on the study website, www.LEOcohort.org.

There is still much more to learn about improving lymphoma survivorship. To support this important research, the LEO study relies on questionnaires occasionally sent to participants to collect information such as updates on health status and well-being. It is important to hear from all study participants. Thank you for taking the time to give us an update!

Follow this link to the Survey:
Take the Survey

Or copy and paste the URL below into your internet browser:
https://src.co1.qualtrics.com/jfe/form/SV_23TevJ7CC6cpSyp?Q_DL=QHd7CjLidP7I0IF_23TevJ7CC6cpSyp_MLRP_73bmCCd2CAP6LC5&Q_CHL=email&timepoint=0_5

All information will be kept strictly confidential and will not become part of your medical record. If at any time you have any questions concerning this research study, please do not hesitate to contact us at 1-800-610-7093.

Again, please accept our sincere gratitude for your contributions. Your participation makes this research possible.

Sincerely,
The LEO (Lymphoma Epidemiology of Outcomes) Research Team

LEO Lymphoma Epidemiology of Outcomes

Due to the generosity of individuals like you, we can continue to investigate ways to improve the experiences and outcomes of people with lymphoma. **Thank you** for your continued dedication to the Lymphoma Epidemiology of Outcomes study.

Want to learn more?

Visit our website
www.leocohort.org
or
Contact us at
leocohort@mayo.edu