BIOPRESERVATION AND BIOBANKING
Volume 9, Number 3, 2011
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DOI: 10.1089/bio.2010.0032

ORIGINAL ARTICLES

A Pilot Study of the ELFE Longitudinal Cohort: Feasibility and Preliminary Evaluation of Biological Collection

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Etude Longitudinale Française depuis l'Enfance (ELFE) will be a national French cohort of 20,000 children followed from birth to adulthood. Biological samples will be taken at birth to evaluate the fetal exposition to several substances. A pilot study was carried out in October 2007 to test the preanalytical factors that affected sample quality. A variety of fractions were collected by the midwife after delivery from different blood collection tubes. Options in the collection process were 2 daily transports of samples, centralized and standardized processing methodology, and storage of multiple aliquots in liquid nitrogen or at −80°C. We analyzed preanalytical factors that could have affected coagulation and then soluble CD40 Ligand (sCD40L) as a quality control tool for serum quality. Cord blood and urine were collected from 82% and 84% of women, respectively, who agreed to be followed up in the ELFE project. The use of syringe was the main factor correlated with coagulation (relative risk: 2.79 [1.47; 5.31], $P < 0.01$). Maternity unit status was also associated with coagulation (RR: 1.48 [1.03; 2.13] in a private maternity unit vs. a public maternity) as well as time between collection and centrifugation (RR 1.03 [1; 1.07] when time between collection and centrifugation increases from 1 h). There were no extremely low sCD40L values indicating extreme exposures to room temperatures. This first evaluation study allowed us to stress the importance of carefully recording all potentially critical preanalytical variables that might be used at a large-scale level.

Introduction

Etude Longitudinale Française depuis l'Enfance (ELFE) is a nationally representative cohort of 20,000 children scheduled to be launched in France in 2011. ELFE will take a multidisciplinary approach and aims to assess the impact of environmental exposures and socioeconomic and familial factors on children's development, health, and behavior. Children will be followed from birth through to adulthood over a period of 20 years.[1] Inclusion in the cohort will be based on an initial enrolment interview of the mother when the child is born, so that we can obtain retrospective data on exposures during pregnancy and then a prospective follow-up of the child. The recruitment of newborns precludes real-time collection of samples and data during pregnancy, although fetal exposures affect the future health of the child.[2–5] The assessment of the prenatal environment will rely on the interview with the mother and the collection of biological samples at the child's birth.[4,6]

A pilot study was carried out in October 2007 to apply and evaluate methods for data and sample collection of cord blood, maternal urine, breast milk, and maternal hair. The preanalytical conditions (the different steps from collection to storage) that are known to be responsible for more than 60% of laboratory errors ought to be considered.[7] Researchers need to be aware of the various preanalytical factors to optimize accuracy, reproducibility, and comparability of research results, and so the handling details of the samples should be documented and preanalytical conditions validated by a reliable quality control method.[8] The objective of this report was to describe (1) the acceptability of the biological sample collection by both the mothers and the healthcare teams and (2) the preanalytical factors that affected sample quality for blood and serum.

The protocol for collection, processing, and archiving of biological samples was developed through a consultation of the scientific community involved in the ELFE project. ELFE has to provide resources to support a wide range of future scientific projects. The operating procedures tested in the pilot phase concerned sample collection, temporary storage temperature and transport to a central processing facility, processing, aliquoting, and long-term storage. We then an-

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alyzed the effect of various preanalytical factors (namely transport, collection material used, and status of maternity unit) on coagulation. We measured a storage-associated quality control biomarker in serum from cord blood. This is an indicator that the samples meet a minimum of pre-analytical specifications and are suitable for a large range of downstream biological analyses.

Materials and Methods

Population

The pilot survey included all children born in hospital maternity units from October 1–4, 2007 in the Seine Saint Denis district and Rhone-Alpes region in France. For practical reasons, the survey was restricted to single or twin births. Two to 3 days after delivery, mothers were invited to sign a consent form explaining the general aims of the study and why we were collecting biological samples. Consent forms for biological samples and data collection were dissociated. The participants were given the right to withdraw from the study at any time. Seventy-five percent of the maternity units contacted ($n = 30$) agreed to take part in the pilot study. The acceptance rate of mothers who were invited to participate in the study was 54% ($n = 300$). Ninety percent of mothers who agreed to participate in the study also agreed to provide biological samples, and biological samples were collected for 80% of this population (10% could not be collected because staff had too much to do during delivery of the child). The sample collection had received ethical approval in June 2010.

Sample collection, processing, and storage

The ELFE biobank has been set up to establish a long-term repository of biological material, to support a wide range of present and future scientific questions. Part of the biobank will be stored for further research. A multidisciplinary approach was taken to develop a protocol for the collection, processing, and archiving of samples, rather than focusing on specific downstream analyses. A variety of fractions (serum, plasma, white and red cells, whole blood) were collected from 2 different blood collection tubes (K_2-EDTA spray-dried tubes and serum tubes with clot activator coating), which allow analysis of a wide range of biomarkers. We opted for 2 daily transports of samples, centralized and standardized processing methodology, and storage of multiple aliquots in liquid nitrogen or at −80°C. A unique barcode was attributed to each sample by the central processing facility; the barcode linked each blood collection tube with the unique participant-identifier number to prevent mislabeling of samples or sample mix-ups when transferred from clinical to research staff. A midwife collected cord blood (expected volume: 20 mL) and urine (expected volume: 200 mL) in the delivery room and stored them at 4°C in the maternity unit. The K_2-EDTA blood collection tubes were inverted 10 times to mix the anticoagulant with the whole blood. Two or 3 days later, another midwife explained the project, obtained a consent form, and collected breast milk (expected volume: 5 mL) and maternal hair from the mother in the maternity unit. Mothers were asked to collect and freeze their breast milk at 1 month later at home (expected volume: 100 mL) and send it to the laboratory by mail in a special flask at ambient temperature. Whole blood and urine were maintained at 4°C in the maternity unit for a maximum of 24 h prior to centrifugation (for blood) and 48 h before storage. The blood in the K_2-EDTA tube was then sent to 1 of 2 central facilities located in blood transfusion centers (EFS), where it was centrifuged immediately on reception at 2500 *g* for 10 min. The EFS centers coordinated transport (twice a day in temperature-controlled shipping boxes to central processing platforms), processing, aliquoting, and freezing of the samples.

The different samples from each individual were aliquoted into 0.5 or 1 mL aliquots for long-term frozen storage. Hematological parameters complete blood count (CBC) were assessed as samples arrived at the central laboratory to streamline processing and minimize quality control issues. Different blood fractions were separated by centrifugation at 2500 *g* for 10 min at 4°C. The aliquots were stored at −80°C in cryovials.

Analysis of various preanalytical factors

Cord blood coagulates easily (fetal hematocrit index is high at about 50%) and the way the samples were collected was probably suboptimal in the delivery context because of the high workload of midwives. We performed an analysis of several factors that could have affected the risk of coagulation: the material for the cord blood sampling (needle extraction or collection of dripping blood), the technicity level of the maternity unit (from level 1 for lowest to level 3 for highest), technical infrastructure available according to the technical level of the maternity (with level 1 for maternity with no neonatology service, level 2 for maternity with a neonatal unit, and level 3 for maternity with a neonatal intensive care unit), the status of the maternity unit (private or public), the filling level of the blood collection tube, the duration of transport [in particular, the time between delivery and cord blood collection (H0), the time between cord blood collection and reception at the processing center (H1), and the time between cord blood collection and centrifugation (H2)]. As the distributions of H1 and H2 were not normal, log transformations were used. The nonnormal distributions could be explained by different workflows in the different maternity units. We performed a 2-sample Wilcoxon test. We used a logistic or linear regression model to assess the association between these factors and the presence of a clot in the sample.

Analysis of sample quality of cord blood serum

CD40L is a protein that has shown a high sensitivity to temperature fluctuations in previous studies and can be used as a quality control tool.[9] This biomarker decreases to a level distinguishable from the lowest level that is naturally occurring in response to specific variations in sample handling conditions.[10] A quantitative sandwich enzyme immunoassay (Quantikine Human sCD40 Ligand Immunoassay kit; R&D Systems) was used according to the manufacturer's instructions. This assay was based on CD40L-specific polyclonal capture with detection antibodies and colorimetric detection at 450 nm. sCD40L concentrations were calculated according to the standard curve generated. Detection of sCD40L concentration below 4.3 ng/mL indicated exposure to at least 48 h at +20°C ($P < 0.025$). Using a cutoff provides the most sensitive and specific way of detecting "out of specifications" serum samples. The reported method provides a sensitivity of 97.5% for all samples whose value is higher than 6 ng/mL

Table 1. Description of Aliquots of Blood, Urine, and Breast Milk Samples

Blood collection tubes	*Biospecimen*	*No. of subjects*	*Mean no. of aliquots*	*Volume (mL)*	*No. of aliquots*	*Rate of successful collection (%)*
	Hair	269	—	—	254	89
K_2 EDTA (6 mL)×2	White cells	205	2.9	—	559	82.5
	Red cells	211	3.7	—	739	
	Plasma	211	3.3	0.5	653	
	Whole blood	220	6.6	0.5	1461	
Serum tube (10 mL)×2	Serum	247	9.8	0.5	2228	
Trace element tube (6 mL)×1	Serum trace element	189	3.1	0.5	562	
	Urine	252	10.8	10	2539	84
	Breast milk (collected in maternity unit)	126	9.8	0.5	1134	42
	Breast milk (collected at home)	46	15	10	636	15

and a specificity of 97.5% for all samples whose value is below 4.3 ng/mL (values between 4.3 and 6 ng/mL correspond to the gray zone).

Results

Collection of biological samples

In the pilot study, we recruited 301 women out of 571 newborns in 28 maternity units. Cord blood and urine were collected from 82% and 84% of women, respectively, who agreed to participate in the ELFE project. The acceptance for cord blood donation and urine donation ranges from 50% to 100% and from 36% to 100%, respectively, according to the maternity unit (Table 1).

The pilot study included 2 processing centers, 1 in each of the 2 districts. They received a total of 1976 specimens, and the different specimens from each mother were processed into 29 aliquots of 0.5 mL of whole blood, 11 aliquots of 10 mL of urine, 10 aliquots of 0.5 mL of breast milk collected in the maternity unit, and 15 aliquots of 10 mL of breast milk collected at home for long-term frozen storage. About 20% of the blood samples were coagulated ($n=90$) upon reception and less than 12% arrived in the laboratory more than 24 h after collection in the maternity unit. The median time between collection and centrifugation was 21.2 h (interquartile range: 15.9–25.8 h). Whole blood was tested for a standard range of hematological parameters (red and white cell counts). The median delay between centrifugation and freezing was 4.4 h, with a minimum of 0 and a maximum of 14 h.

Analysis of various preanalytical factors

We studied the association between specific collection and processing factors and the coagulation status of the samples: the status of the maternity unit, the collection materials used, and the transportation delay. A great variation of coagulation according to the maternity unit was observed (coagulated samples proportion ranged from 0% to 100%). Coagulation was more frequent in samples from private maternity units than from public ones (27.8% vs. 16.5%); coagulation was also more frequent when a needle was used for collection (41.7% vs. 16.0%) (Table 2).

Table 3 describes the time between collection and centrifugation according to coagulation status. The average transport time of coagulated samples was higher than that of noncoagulated samples (median: 20.9 for noncoagulated samples vs. 22.7 for coagulated samples).

Table 2. Description of Coagulation Status According to Specific Preanalytical Factors for Cord Blood Samples

		Coagulated		
		N	*%*	*Total*
Category of maternity unit				
Maternity with no neonatology services	1	40	24.1	166
Maternity with a neonatal unit	2	27	16.5	164
Maternities with a neonatal intensive care unit	3	23	19.7	117
Status				
Private		40	27.8	144
Public		50	16.5	303
District				
Rhône-Alpes		50	16.3	306
Seine Saint Denis		40	28.4	141
Needle used for collection				
No		60	16.0	375
Yes		30	41.7	72
Filled vacutainer				
Yes		20	24.4	82
No		20	33.9	59
Unknown		50	16.3	306

Table 3. Description of Coagulation According to Time Between Collection and Centrifugation

Coagulation	*Variable*	*Nb*	*Mean (h)*	*SD*	*Min*	*p25*	*p50*	*p75*	*Max*
No	Time	355	20.9	8.4	3.6	15.0	20.9	25.5	51.7
Yes	Time	90	22.3	7.4	5.6	18.3	22.7	26.4	42.3

	Coefficient	*SD*	*z*	*Pr (>|z|)*	*RR*	*95% CI*
Constant	−1.960	0.312	−6.291	<0.001		
Time (linear relation over 0–20 h interval)	0.032	0.017	1.856	0.063	1.03	[1; 1.07]
District Seine-Saint-Denis and no syringe	Ref					
District Seine-Saint-Denis and syringe	1.026	0.328	3.129	0.002	2.79	[1.47; 5.31]
District Rhône-Alpes and no syringe	0.236	0.333	0.708	0.479	1.27	[0.66; 2.43]
Maternity status: public	Ref					
Maternity status: private	0.392	0.186	2.102	0.036	1.48	[1.03; 2.13]

RR, relative risk.

To study the effects of preanalytical factors on the coagulation, a log-Poisson regression was performed, linking coagulation status to district, needle use, status of maternity, and time between collection and centrifugation. Log-Poisson modeling has been chosen as an alternative to logistic modeling when odds ratio is not a good approximation of relative risks; indeed, coagulation proportions are high and depend on explaining variables, for example, 16% vs. 42% for public vs. private maternities.

This model showed that the use of the syringe was the main factor correlated with coagulation (relative risk: 2.79 [1.47; 5.31], $P<0.01$, for syringe use vs. no syringe use in Seine-Saint-Denis). Maternity unit status and time between collection and centrifugation are also associated with coagulation:

- RR is 1.03 [1; 1.07] when time between collection and centrifugation increased from 1 h.
- RR is 1.48 [1.03; 2.13] when sample was collected in a private maternity unit (public maternity unit was taken as reference).

No association between coagulation and time at room temperature spent between reception and centrifugation was observed.

Analysis of sample quality in cord blood serum

sCD40L was analyzed in 271 serum aliquots. Mean value was 15.62 ng/mL (SD 4.52) and range was 3.8–27.3 ng/mL. No significant difference was observed between serum samples prepared from coagulated and noncoagulated blood specimens. A significant correlation was observed between sCD40L levels and delay between centrifugation and storage ($R=0.15$, $P=0.015$).

We did not observe any extremely low sCD40L values, indicating extreme exposures to room temperatures. Only 6 serum samples exhibited borderline sCD40L concentrations of 3.8–4.9 ng/mL, suggesting a possible 48 h exposure to +20°C. The 6 serum samples exhibiting borderline sCD40L concentrations had time between collection and centrifugation (>18 h) and/or between collection and storage (>21 h).

Discussion

The response rate for biological collection was satisfactory for cord blood and urine (more than 80% of women) but lower for milk collection. This can be explained because the rate of breast feeding was 68% ($n=204$) at delivery and 42% ($n=126$) accepted to collect milk for research purposes. This rate decreased to 48% ($n=144$) at 1 month postdelivery and 15% ($n=46$) accepted to collect it by then. No quality biomarker for preanalytical validation of milk or urine is available to our knowledge. Therefore, we focused our analyses on sample quality in serum and blood.

Biospecimen characterization and preanalytical validation is one of the major challenges facing modern biobanking.[11] In this pilot survey, we performed a first evaluation of the collection, transport, processing, and storage methods of samples for the ELFE project. The average time to storage (from collection of blood and urine to storage at ultra-low temperature) was 25 h, which is similar to other large-scale studies (for example, the UK Biobank has an average time to archiving of 23 h) even if blood samples were not centrifuged in the clinic setting before shipping to the central laboratory, as it was done in UK Biobank, for instance.[12] This process was a compromise between feasibility on a large scale and the requirements of many scientific investigations. We centralized and standardized processing in a single blood transfusion center to ensure a high-quality data trail. We used 2 geographically separate repositories. The secure data audit trail was maintained using quality management system ISO 9001–2008.

The use of health service infrastructure to collect biological samples at the maternity unit for epidemiological purposes is a challenge. Indeed, the conditions under which biological samples are collected and processed in the course of providing health care in maternity units could be inadequate for the measurement of less-stable biochemical markers of epidemiological interest, such as cytokines.[13] The procedures used for sample collection, processing, and storage have a major impact on the future scientific usefulness of ELFE.[14]

Thus, about 20% of the blood samples were coagulated upon reception. This could be explained by practical reasons in the delivery room, such as immediate or delayed cord clamping, or other factors within control because of different conditions in the maternities (eg, high workload of staff); these could also be reasons for partially filled tubes as well as the lack of sufficient volume of available blood. These differing conditions between maternity units could explain the association between hospital private status and higher rate of coagulation. Private hospitals are less research oriented and have not the same academic background or practices as public maternity units. Moreover, coagulation was also more

frequent when a needle was used for collection, probably because physiological adaptation to delivery trauma induces hypercoagulability in the newborn's blood.[15] Consequently, all mechanical factors such as the syringe shearing force and mechanical pressure through aspiration induce platelet and tissue factor activation and coagulation. This activation is not induced by natural dripping of blood.

A quality control study was performed to test the robustness of the pilot sample handling and storage protocol, by measuring sCD40L, which is an indicator of the delay of exposure of serum to room temperature. It has been previously shown that the sCD40L levels in cord blood are lower than in peripheral blood, which further consolidates the results of this study in terms of cord blood–derived serum integrity.[16]

Finally, it should be kept in mind that although serum sample integrity was successfully assessed on the basis of sCD4OL assay, novel quality control tools (such as surveillance of freeze–thaw cycle temperatures) need to be developed to assess other types of preanalytical variation and to allow a more complete characterization of large cohort samples.

In this pilot survey, we validated the collection, transport, processing, and storage methods of samples for the ELFE project. A quality control study was performed to test the robustness of the pilot sample handling and storage protocol by measuring sCD40L, which is an indicator of the delay of exposure of serum to room temperature. Studies of serum sample integrity need to be more investigated, as preanalytical validation is one of the major challenges facing modern biobanking. In the mean time, it can be highly recommended to carefully record all potentially critical preanalytical variables, with tools such as a Standard PREanalytical Code (SPREC).[17]

Acknowledgments

ELFE pilot was a joint study between The National Institute for Demographic Studies (INED), The National Institute for Health and Medical Research (INSERM), the Institute for Public Health Surveillance (InVS), the National Institute for Statistics and Economic Studies (INSEE), the Ministry of Health, the Ministry of Employment, the National Family Allowance Office (Cnaf), the Ministry of Research, the Ministry of Environment, the National Social Science Data Committee (CCDSHS), the Complementary Insurance Organization for Educators (MGEN), and the Ministry of Culture. The authors acknowledge all the members of the ELFE team and all the members of the project, particularly the theme managers. The authors are grateful to midwifes and hospital maternity units who participated in this study.

Author Disclosure Statement

No competing financial interests exist.

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Received 20 December, 2010/Accepted 27 March, 2010