EBioMedicine 12 (2016) 32–33

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Commentary

Plasma Protein Timings: Relative Contributions of Storage Time, Donor Age and Donation Season

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ARTICLE INFO

Article history:
Received 4 October 2016
Accepted 4 October 2016
Available online 5 October 2016

Keywords:
Biobank plasma variable storage time age season

Development of the field of biorepository and biospecimen science has been shown to be a crucial tool for the development of biomarkers for health and disease and the development of testable hypotheses in clinical research (Vaught et al., 2011).

The times when bio-specimens were tracked through laboratory notebooks and kept in a few freezers are over. Advances in epidemiology and omics science have led to a growth in national and international biobanks for regional healthy populations, or disease-specific populations of individuals to support large-scale research resources (Hallmans and Vaught, 2011).

Specifically, the ease of processing blood derived plasma has favored the global spread of plasma biobanks. Plasma specimens lack the confounding factors associated with the cell release of proinflammatory cytokines and metabolites during clotting (Lee et al., 2015), which has resulted in plasma being preferred over serum for protein abundance analyses (Stenemo et al., 2016). Long-term stability of collected, stored plasma specimens is essential for longitudinal studies and for retrospective studies with specimens which have been for stored different periods. Large efforts have been made to collect carefully matched case and control cohorts, adjusting for non-disease related factors such as age and gender, or risk factors such as body mass index, weight and smoking. Also, plasma components have been found to change with storage time before separation and freezing, freezer storage time and number of freeze-thaw cycles (Paltiel et al., 2008; Harish et al., 2011). While donor individual age has been shown to affect the plasma protein levels in many studies (Liu et al., 2015), natural variables such as, time of day, weekday and general intra-individual variation which are also known to affect the variability and reproducibility of specimens stored in biobanks (Stenemo et al., 2016), have not been previously studied for their contribution to the variability in large cohorts of specimens from biobanks. From monozygotic and dizygotic twin studies, it has been demonstrated that the levels of different plasma proteins show vastly different patterns of abundance variability among humans, and that genetic control and longitudinal variation affect protein levels and biological processes to different degrees (Liu et al., 2015). However, the compounded heterogenic nature of genetic and environmental variables as well as changes in the circadian/seasonal cycles of protein levels affecting human plasma specimens available in biobanks has not allowed, as yet, the determination of the specific weight of these parameters in the context of protein stability in long-term stored plasma specimens.

In this issue of EBioMedicine, Enroth and colleagues (Enroth et al., 2016-in this issue) present a comprehensive study that analyzes the individual contribution of factors related to the collection and long-term sample storage in a highly homogenous group of Northern European subjects (Karlsson et al., 2006). Taking advantage of the sampling strategy of the Västerbotten Intervention and Mammography screening programs in northern Sweden (http://epi.grants.cancer.gov/Consortia/members/northsweden.html) at the Umeå Biobank, this study intended to disentangle the effects of freezer storage time and individual age in a relatively homogenous cohort of subjects. The study analyzed the levels of 108 proteins, as assessed by the highly specific multiplex assays which use antibody-mediated proximity paired oligonucleotides and PCR amplification (Lundberg et al., 2011). They selected 380 periodically-drawn specimens from 106 healthy local females with ages of 40, 50 and 60 years from 1988 through 2014 from the Västerbotten Intervention Program and from the Mammography Screening Program for females in the 50 to 69 age group since 1995. All female specimens were collected at specific ages in a concrete population with relatively small diversity level. In a nutshell, this study provides evidence that in addition to the storage time, which affected 18 proteins and explained 4.8–34.9% of the observed variance, the chronological age at sample collection, and to a lesser degree, the season of collection were responsible for part of the variance. In fact, the chronological age at sample collection after adjustment for storage-time affected 70 proteins and explained 1.1–33.5% of the variance, very similar to the effect of storage time, while the seasonal variation in specimens collected in a region with a

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2016.08.038.
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http://dx.doi.org/10.1016/j.ebiom.2016.10.005

large variation in daylight hours between winter and summer, affected 15 proteins and explained up to 4.6% of the variance seen in protein abundance levels after adjustment for storage-time and age. This finding is also interesting since the town of Umea in Sweden is located at 63 degrees North and receives an average of 60 daylight hours per month during the winter months (November to March) compared to over 260 hours per month during summer (June to August). Skin radiation affects the circulating levels of plasma proteins (Reichert et al., 2015), and sunlight hours may viewed as a proxy variable to other seasonal changes such as plant blooming or increasing pollen levels which in turn triggers the immune system, or various forms of changes in lifestyle such as seasonal intakes of food and levels of physical activity. In this manuscript, Enroth and colleagues (Enroth et al., 2016-in this issue) present three examples of significant changes in protein levels which were illustrative of this effect (TGFβ1, HSP-27, IL-20RA) where levels were significantly lower during the summer months (except July when no specimens had been collected). While this variation had already been reported for other tests like serum cholesterol (Ockene et al., 2004), this study provides additional information on the actual weight of the effect of collection season on potential biomarker analyses and shows that although the season of the year does matter, its weight compared with the storage time and donor chronological time is much lesser.

While this study demonstrates the existence of a large variance introduced by storage time, and underscores the inclusion of storage time as an important variable in future epidemiological studies, this study also provides for first time information on the actual impact of the chronological age of the donor and of seasonal/month variation.

This study has profound implications for the design of case-control or cohort longitudinal studies and further emphasizes the need for accurately chronologically-matched controls and in some cases, the need to account for seasonal differences collection that may significantly modify the levels of specific plasma proteins.

Disclosure

The authors declare no relevant conflicts of interest.

Acknowledgements

The authors want to thank Ms. Margaret O'Leary for her editorial assistance.

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