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Gene expression profiles are different in venous and capillary blood: Implications for vaccine studies

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ABSTRACT

Background: Detailed analysis of the immunological pathways leading to robust vaccine responses has become possible with the application of systems biology, including transcriptomic analysis. Venous blood is usually obtained for such studies but others have obtained capillary blood (e.g. finger-prick). Capillary samples are practically advantageous, especially in children.
Methods: The aim of this study was to compare gene expression profiles in venous and capillary blood before, 12 h and 24 h after vaccination with 23-valent pneumococcal polysaccharide or trivalent inactivated seasonal influenza vaccines.
Results: Gene expression at baseline was markedly different between venous and capillary samples, with 4940 genes differentially expressed, and followed a different pattern of changes after vaccination. At baseline, multiple pathways were upregulated in venous compared to capillary blood, including transforming growth factor-beta receptor signalling and toll-like receptor cascades. After vaccination with the influenza vaccine, there was enrichment for T and NK cell related signatures in capillary blood, and monocyte signatures in venous blood. By contrast, after vaccination with the pneumococcal vaccination, there was enrichment of dendritic cells, monocytes and interferon related signatures in capillary blood, whilst at 24 h there was enrichment for T and NK cell related signatures in venous blood.
Conclusions: These data show differences between venous and capillary gene expression both at baseline, and post vaccination, which may impact on the conclusions regarding immunological mechanisms drawn from studies using these different sampling methodologies.

1. Introduction

Vaccines are an unrivalled intervention in terms of impact on morbidity and mortality due to infectious disease, but the immunological processes determining protection following vaccination are not fully understood [1]. The use of high-throughput technology allowing different pathways and their interactions to be examined holistically, and subsequent integration and analysis of the data produced has been used to investigate transcriptional changes in response to vaccination in systems biology analyses. Analysis of such changes have identified gene expression signatures that correlated with immunogenicity following yellow fever and influenza vaccination [2–4].

The comprehensive data thus generated provide a novel approach to interrogate the molecular mechanisms underlying host responses to infection and vaccination and provide a new tool in the development of vaccines. One of the most cost-effective methods of analysing changes in the transcriptome is the use of RNA micro-arrays. Transcriptional data provide a snapshot of the genome wide expression profile at a specific point in time allowing conclusions about the molecular host responses to an immunological stimulus to be drawn if an analysis of global gene expression changes is performed before and after vaccination [2]. While useful, this approach produces thousands of data points, which can make extraction of coherent information difficult. Gene set enrichment, where catalogues of annotated sets of genes are used to interrogate the data, has allowed the extraction of biologically meaningful and objective information, leading to advances in understanding of changes in response to vaccination [5].

Protocols for the exploration of transcriptional changes have not been standardised between studies, sample types, timing and processing. Some studies have used whole blood from venepuncture whereas others use PBMCs from the same type of venous sample and these samples differ significantly [1–3,6]. It has been suggested that whole blood may result in reduced

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detection sensitivity, and that prolonged handling of PBMC samples is associated with artifactual changes. Capillary blood has been used as a more convenient sample to obtain, particularly in studies of children. This means that studies using different sample types and processing and analysis methods cannot easily be compared. As gene expression data tend to be susceptible to sample processing and handling, sampling methods may impact on the conclusions drawn from transcriptomic studies, leading to potentially redundant functional experiments.

It is thus important to know whether venous and capillary samples produce results that are comparable. However, the difference in gene expression between capillary and venous blood samples has not been previously studied. In the present study we directly compared data derived from samples obtained simultaneously from individuals by venepuncture (venous) and fingerprick (capillary), which are publically available as a resource from the Gene Expression Omnibus (GEO) database, and investigated differential gene expression before and after vaccination, between these methods [7].

2. Methods

2.1. Data source

The raw sample and control data used by Obermoser et al. were downloaded from the Gene Expression Omnibus (GEO) database (Accession: GSE30101) [7].

2.2. Study design and participants

The study population has been described in detail previously [7]. Briefly, healthy adult volunteers aged 18–64 years received the seasonal influenza vaccine (Fluzone, Sanofi Pasteur), pneumococcal polysaccharide vaccine (Pneumovax23, Merck) or saline control (Table 1). Blood was obtained at various time points, including four time points where both fingerprick (capillary) and venepuncture (venous) samples were taken simultaneously from the same subject (7 days pre-vaccination [−7 d], at the time of vaccination [0 d], and 12- and 24-h post-vaccination [12 h, 24 h]), providing an opportunity to compare transcript expression between the two sample types.

2.3. Data processing

Microarray data were background subtracted, and a force positive modification used so that a $\log_2$ transformation could be performed, followed by a robust spline normalisation of the data (R package Lumi) [8]. The data were filtered to only include probes that were detected in greater than 65% of all the samples in the analysis, to exclude inconsistently detected probes ($p < 0.05$; R Package Genefilter).

2.4. Data analysis: fold change

Paired analysis was conducted to compare transcript expression in capillary and venous blood derived from the same individual. The baseline samples (−7 d, 0 d) from all study groups – saline, influenza and pneumococcal vaccine groups were analysed. The pre- (0 d) to post-vaccination fold changes were then tested using a paired *t*-test between sample types. Comparison of venous against capillary was done using a paired *t*-test, and pre-, post-vaccination changes were calculated using a linear regression. The changes were compared between venous and capillary with a paired *t*-test. Only samples with a pair were tested; un-paired samples were not analysed. These tests were fit using the lmFit and eBayes functions (R Package Limma) [9]. A p-value of $\leqslant 0.001$ and an absolute fold-difference between venous and capillary or between pre- and post-vaccination in expression of greater than 1.25 was used to select differentially expressed transcripts. To find total genes differentially expressed at baseline, the total numbers from −7 d and 0 d were added and replicates removed, whilst transcripts consistently differentially expressed were calculated using genes differentially expressed at both −7 d and 0 d. To compare numbers of transcripts between venous and capillary samples, a McNemar's test was performed. Bland-Altman plots containing all genes differentially expressed post-vaccination were used to examine whether the transcript expression changes were comparable when measured in venous and capillary blood.

2.5. Pathway analysis

Pathway analysis was performed on differentially expressed transcripts using the publically available analysis tool InnateDB [10]. Pathways were considered differentially expressed if the false-discovery rate was <0.05. Two pathway analyses were performed at baseline: an analysis using total genes differentially expressed between venous and capillary blood, and a split analysis using only the genes higher in either venous or capillary blood.

2.6. Gene set enrichment analysis

Gene Set Enrichment Analysis (GSEA) was performed using R. Gene lists were ranked by log2 FC and analysed for enrichment of blood transcriptional modules (BTMs) previously described by Li et al. [11,12]. Nominal p-values for Normalised Enrichment Scores (NES) were calculated using 1000 random gene set iterations and corrected for multiple testing using the Benjamini-Hochberg (BH) method. Gene lists used included the comparisons of capillary against venous at baseline (day 0) and capillary against venous fold change after vaccination.

3. Results

3.1. Investigation of variability in expression

To examine the variability in expression levels at a global level between venous and capillary samples, numbers of detected probes, mean probe intensity distributions, and distributions of variance were produced (Supplementary figure 1). These showed that while there was a higher number of probes detected on average in venous than capillary samples, variance and mean

Table 1
Number of participants included for each vaccine and sample type and each time point, including overlap between sample groups.

Time	Sample	−7 d	0 d	12 h	24 h
Influenza vaccine	Venous	12	18	6	18
	Capillary	6	6	6	6
	Both	6	6	6	6
Pneumococcal vaccine	Venous	12	16	6	16
	Capillary	6	6	6	6
	Both	6	6	6	6