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Gene expression patterns in multiple organs in experimentally induced *Staphylococcus aureus* sepsis in pigs

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Introduction

Animal research in sepsis needs analytical tools that can capture and exploit the complexity of the condition.

To summarise the disease progression in a porcine model of severe *Staphylococcus aureus* sepsis, we used principal component analysis (PCA) to identify early dynamic expression patterns of 34 selected genes in liver, lung, and spleen tissue.

Materials and methods

Seventeen infected pigs[1,2] were euthanised at the following time points *post infection* (PI): 6 h (n = 3), 12 h (n = 3), 24 h (n = 3), 30 h (n = 1), 36 h (n = 2), and 48 h (n = 5).

Five healthy controls were managed in parallel.[1,2]

Gene expression of 34 genes related to acute inflammation and haemostasis was measured in liver, lung, and spleen by quantitative real time PCR (Figure 1).

The data matrix of 22 samples and 102 (34 x 3) variables were log-transformed, scaled to unit variance, and subjected to PCA.

Results

Three (PC1 to PC3) distinct dynamic response patterns were identified:

PC1:
Hepatic positive and negative acute phase genes were the main influencers of a protracted pattern induced between 12 to 48 h of infection which explained 23% of the total variation in the dataset (Figure 2A and C).

PC2:
An acute pattern distinguished infected pigs from controls already after 6 h and peaked around 12 h PI. After 30 h to 48 h, pigs had either reverted back to basal levels (n = 7), or below basal levels (n = 2) (Figure 2A). This pattern explained 14% of the total variation and was influenced by a systemic (non-organ-specific) mixture of pro-inflammatory, anti-inflammatory and haemostatic genes (Figure 2C).

The two pigs with low PC2 levels had suffered from overt disseminated intravascular coagulation when euthanized.[3]

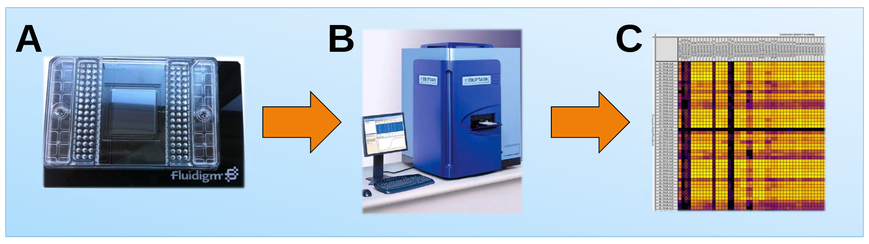
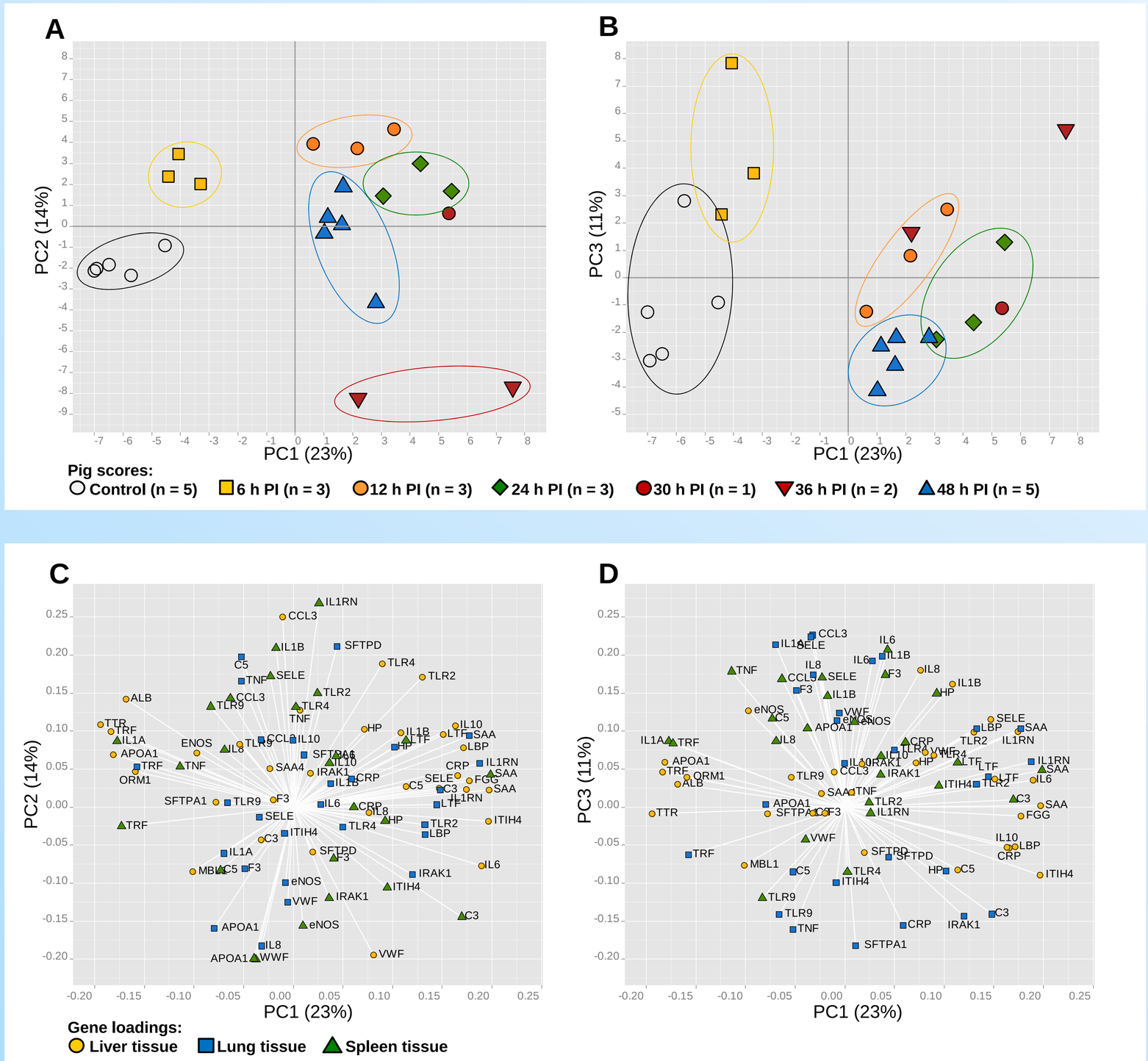


Figure 1
A high-throughput qPCR platform was used in this study, which measured 48 samples x 48 primers per reaction.
A) a 48.48 Dynamic Array (Fluidigm); **B)** Biomark HD system (Fluidigm); **C)** a heat-map overview of gene expression data (cycling thresholds).

Figure 2
Pigs (scores) and genes (loadings) in PC1-PC2 (**A** and **C**) and PC1-PC3 space (**B** and **D**).
In **A** and **B**, the time-wise evolvement is highlighted by encirclement of animal groups according to their time of euthanisation



PC3:
A per-acute pattern, influenced mainly by pulmonary pro-inflammatory genes (explaining 11% of the total variation), was induced in infected pigs at 6 h PI, while at later time points, most pigs had moved towards basal levels (Figure 2B and D).

Conclusion

Multivariate analysis (PCA) identified three temporally distinct patterns in gene expression data from liver, lung, and spleen tissue:

1) Pulmonary inflammation was rapidly induced, followed by
2) transient induction of a generalised inflammatory and haemostatic response, and
3) initiation of the hepatic acute-phase response.

Acknowledgements

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