Altered mRNA Expression Due to Rectum Perforation in a Porcine Model – A Pilot Study

Ioannis Oikonomakis (ioannis.oikonomakis@regionorebrolan.se)
Department of Surgery, Faculty of Medicine and Health, Örebro University, Örebro

David Brodin
Department of Biosciences and Nutrition, Karolinska Institute, Stockholm

Tal M. Hörer
Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine and Health, Örebro University, Örebro

Per Skoog
Department of Vascular Surgery and Institute of Medicine, Department of Molecular and Clinical Medicine, Sahlgrenska University Hospital and Academy, Gothenburg

Jenny Seilitz
Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine and Health, Örebro University, Örebro

Kristofer F. Nilsson
Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine and Health, Örebro University, Örebro

Adrian D. Meehan
Department of Geriatrics, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

Kjell Jansson
Department of Surgery, Faculty of Medicine and Health, Örebro University, Örebro

Research Article

Keywords: colorectal surgery, anastomotic leak, gene expression analysis, microarray

Posted Date: March 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-244139/v1

Abstract

Background

Anastomotic leakage is the most serious and unwelcome complication in rectal surgery. It has a great impact on postoperative morbidity and mortality. In this pilot study changes of mRNA expression in blood were analyzed in an animal model designed to imitate anastomotic leakage.

Materials and Methods

Twelve pigs were randomized into two groups. A control group and an experimental group, there an iatrogenic rectal perforation was performed. The changes in the mRNA expression were studied four hours after the perforation. Microarray analysis was performed using the Gene Chip whole porcine genome array.

Results

19 124 mRNA genes were investigated. Significantly increased levels of genes with a fold change (FC) over 2 were found, including 276 genes coding for an unknown protein and 48 mRNA coded for a known protein. Eleven of the genes which coded for a known protein were highly up-regulated with an FC >4.

Conclusion

Eleven known genes were consistently up-regulated after the rectal perforation. These genes were mainly involved in inflammatory response, intracellular signaling and cell membrane regulation. Their corresponding proteins could potentially be clinical biomarkers of anastomotic leakage and should be evaluated in further clinical studies.

Background

Surgical complications in colorectal surgery are common and feared, the diagnosis is often made late in the process due to the fact that there are no conclusive laboratory tests or X-ray examinations to determine this complication early in the process. CRP is the most commonly used laboratory test but it is clinically reliable first after 4-5 postoperative days. CT scan with anal enema can often not detect a leak in the early postoperative stage (1-4). Intraperitoneal microdialysis (IPM) has been used in studies and has shown promising results but has not been used in clinical care for early diagnosis of anastomotic leakage (5-11). Cytokines are sensitive biomarkers for inflammation, but not specific for the detection of AL (12-17). mRNA analysis offers a new pathway in the search for laboratory markers specific for detecting an anastomotic leakage. mRNA links genetic information from DNA to the ribosome in order to facilitate gene expression. RNA polymerase transcribes primary transcript mRNA into mature mRNA. Mature mRNA is then translated into a protein in the ribosome. mRNA genetic information is a sequence of nucleotides, which are arranged into codons each consisting of three base pairs. Each codon encodes for a specific amino acid, except the stop codons, which terminate protein synthesis. Two other types of

RNA contribute to protein synthesis, tRNA contributes in the recognition of the codon and rRNA plays a central role in the construction of protein chains (18). There are now commercially available microarrays for both human and animal specimens that can detect the expression of up to 20–30,000 genes (19-24). In this pilot study, alterations of mRNA expression in blood samples due to a rectal perforation were compared between an experimental group and a control group. The aim of this study was to identify a biochemical marker for the early detection of AL by analysing the whole blood changes in gene expression in response to rectal perforation using a whole genome porcine microarray by inductive strategy.

Materials And Methods

Animals

In this study, 12 healthy three-months-old domestic pigs (a crossbreed between Swedish country breed, Hampshire and Yorkshire) of both sexes were used, with mean body weight 28 kg (21-37). The pigs were housed at room temperature at a farm, with free access to standard porcine fodder before the experiment. They were kept in a 16-hour day and 8-hour night cycle. The experiment was approved by the Regional Animal Ethics Committee in Linköping (Dnr 174-3). The study was conducted in accordance with the guidelines of the European Union for the protection of animals used for scientific purposes. The animal experimentation in this study is reported according to the ARRIVE guidelines (25).

Anesthesia, fluid administration, ventilation and euthanasia

As premedication at the farm the pigs were given Azaperone (200 mg, i.m.; Elanco, Herlev, Denmark). On arriving at the laboratory, anesthesia was induced by Tiletamine (6 mg kg^{-1}, i.m.; Virbac, Kolding, Denmark), Zolazepam (6 mg kg^{-1}, i.m.; Virbac) and Azaperone i.m. (4 mg kg^{-1}). In addition, Atropine (1.5 mg, i.m.; Mylan, Stockholm, Sweden) was given to prevent excessive salivation. The animals received two peripheral catheters (1.1 mm, Venflon Pro Safety, BD, Helsingborg, Sweden) in auricular veins. Propofol (1-2 mg kg^{-1}, i.v.; Fresenius Kabi, Uppsala, Sweden) was given if needed. The animals were orally intubated in the prone position with a 6-mm endotracheal tube (Covidien, Tullamore, Ireland). Anesthesia was maintained by Propofol (10 mg kg^{-1} h^{-1}, i.v.) and Petidin (1 ml kg^{-1} h^{-1}) applied by motorized syringe pumps (Alaris CC, Cardinal Health, Rulle, Switzerland). The depth of anesthesia was intermittently monitored by pain response. No muscle relaxants were given. Ringer`s acetate (10 ml kg^{-1} h^{-1}, i.v.; Fresenius Kabi) and 10% Glucose with 40 mM sodium and 20 mM potassium (0.5 ml kg^{-1} h^{-1}, i.v.; Fresenius Kabi) were administered by volume pumps (Alaris GP, CareFusion), to substitute for fluid loss. The pigs were ventilated using volume-control ventilation mode (PV 501, Breas Medical AB, Sweden) to achieve an arterial Pco_2 of 5.0-5.3 kPa and Fio_2 was adjusted to maintain arterial Po_2 at 12-18 kPa. The animals were placed on a thermal mattress and covered with a forced-air warming blanket. At the end of the experiment euthanasia was performed with a rapid i.v. injection of 40 mmol potassium chloride (B. Braun, Danderyd, Sweden), and asystole and circulatory arrest were confirmed with ECG and blood pressure recordings.

Surgical preparation and measurements

A 4-Fr introducer was placed in the right carotid artery by open exploration, for the measurement of systemic blood pressure as well as blood sampling. A midline abdominal incision was performed. A 14-Fr Foley catheter was inserted in the urinary bladder and fixed with a purse-string suture. Rectal perforation was performed with a scissor. The perforation was located 8 cm above the anal verge. The diameter of the hole was 3 cm. The midline incision was sutured at the end of the procedure with a continuous suture.

Protocol

Using a sealed envelope system, the animals were randomized before the operation into two groups: 1. An experimental group with rectal perforation and 2. A sham operated control group. After the operation, there was an intervention free period of one hour. Blood samples for mRNA analysis were taken before the laparotomy, and 4 hours after the rectal perforation (Image1).

mRNA analysis

mRNA analysis was performed by Bioinformatics and Expression Analysis, core facility (BEA) , which is supported by the Faculty Board of research at the Karolinska Institute and the Committee for Research at the Karolinska University Hospital,Stockholm, Sweden. Total RNA was isolated from PAXgene Blood RNA tubes with PAXgene Blood RNA Kit standard protocol on a QIAcube, Qiagen. Total RNA quality was assessed by Agilent Technologies 2200 Tapestation and concentrations were measured by NanoDrop ND-1000. Spectrophotometer. Label protocol: 150ng of total RNA was used to generate amplified sense strand DNA targets using Affymetrix WT Plus Kit followed by fragmentation and biotinylation Hybridization protocol: 2.2ug of ss DNA target was hybridized to Porcine Gene 1.0 ST Arrays in Affymetrix Gene Chip Hybridization Oven 645(26). Hybridization, washing and staining was carried out on Affymetrix GeneChip® Fluidics Station 450, according to the manufacturer's protocol. The fluorescent intensities were determined with Affymetrix GeneChip Scanner 3000 7G. Protein identification was performed according to gene databases. (www.uniprot.org, www. geneontology.org)

Statistical analysis

Statistical analysis was performed with unpaired T-test with the assumption that the values were normally distributed, in R-interface software system, version 3.6.1. *P*-values were adjusted for multiple testing using with Benjamini-Hochberg procedure. $P < 0.05$ considered significant.

Results

Preoperatively and under the experiment both groups had stable pulse, blood pressure and urine production. In total, 19,124 mRNA genes were investigated. A significant number of genes were found to

be up regulated and with an FC>2, including 276 genes which coded for an unknown protein and 48 genes which coded for a known protein. Further, eleven up-regulated genes were identified with an FC>4 and these are presented separately in the text below. The majority of the annotated genes are involved in the inflammatory response, regulation of the membrane and intracellular signaling (Table 1).

15242239 is the identification number for the mRNA transcript encoding for the protein TNF-alpha-induced protein 6. It had an FC of 11.7 and was the highest FC measured in this study. It was noted that mRNA in blood for TNF-alpha-induced protein 6 increased over 11 times 4 hours after the rectal perforation (*p*=0.00074) (Figure 1, 2).
15218761 mRNA transcript encoding for the protein potassium inwardly-rectifying channel, subfamily J, member 15. The FC was 9.7, was shown to increase over 9 times 4 hours after the rectal perforation (*p*= 2.8E-05) (Figure 1, 2).
15295924 mRNA transcript encoding for the protein haptoglobin, FC was 6.6 (*p*=0.00021) (Figure 1, 2).
15254582 the mRNA transcript encoding for the protein transcobalamin I (vitamin B12 binding protein, R binder family), FC was 6.4 (*p*=0.01934) (Figure 1, 2).
15287911 mRNA transcript encoding for the protein activin A receptor, type IB, FC was 6.3 (*p* =0.00021) (Figure 1, 2).
15254535 mRNA transcript encoding for the protein membrane-spanning 4-domains, subfamily A, member 7, FC was 6.0 (*p*=0.00136) (Figure 1, 2).
15330079 mRNA transcript encoding for the protein matrix metallopeptidase 1 (interstitial collagenase), FC was 5.8 (*p*=0.0000794) (Figure 1, 2).
15285579 mRNA transcript encoding for the protein S100 calcium binding protein A8, FC was 4.6 (*p*=0.00026) (Figure 1, 2).
15190807 mRNA transcript encoding for the protein lipocalin 2, FC was 4.7 (*p*=0.00021) (Figure 1, 2).
15280666 mRNA transcript encoding for the protein S100 calcium binding protein A9, FC was 4.6 (*p*=0.000342) (Figure 1, 2).
15263686 mRNA transcript encoding for the protein resistin, FC was 4.3 (*p*=0.00106) (Figure 1, 2).

Table 1: Gene expression data. Includes Probe Set Identification Number, FC, p -value and protein description of those 48 mRNA which coded for a known protein.

Probe Set ID	FC	p-value	Protein
15242239	11.701	0.000737	tumor necrosis factor, alpha-induced protein 6
15218761	9.7341	0.0000275	potassium inwardly-rectifying channel, subfamily J, member 15
15295924	6.6565	0.000212	Haptoglobin
15254582	6.3697	0.019335	transcobalamin I (vitamin B12 binding protein, R binder family)
15287911	6.2881	0.000212	activin A receptor, type IB
15254535	5.9668	0,001361	membrane-spanning 4-domains, subfamily A, member 7
15330079	5.7942	0,0000794	matrix metallopeptidase 1 (interstitial collagenase)
15285579	4.8529	0.000257	S100 calcium binding protein A8
15190807	4.7245	0.000212	lipocalin 2
15280666	4.645	0.000342	S100 calcium binding protein A9
15263686	4.2737	0.001065	Resistin
15249538	3.856	0.008008	antileukoproteinase-like
15247299	3.4524	0.005042	antileukoproteinase-like
15263641	3.3756	0.004251	egf-like module containing, mucin-like, hormone receptor-like 1
15275209	3.2573	0.00047	AT-rich interactive domain-containing protein 5A-like
15322017	3.1694	0.0000751	progestin and adipoQ receptor family member 3-like
15248234	3.1559	0.000212	prion protein
15311674	3.0158	0.004586	protein-glutamine gamma-glutamyltransferase K-like
15281489	2.9409	0.000212	bcl-2-like protein 15-like
15285575	2.9376	0.000231	S100 calcium binding protein A12
15247429	2.8959	0.003717	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
15305270	2.795	0.027735	sphingomyelin phosphodiesterase, acid-like 3B
15248514	2.7089	0.014077	thrombomodulin
15270286	2.6795	0.007568	interleukin 1 receptor, type II
15312575	2.5983	0.001713	arginase, type II
15330095	2.4856	0.013268	matrix metallopeptidase 3 (stromelysin 1, progelatinase)
15283223	2.4671	0.0000411	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1
15328685	2.4087	0.005984	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2-like
15328709	2.3975	0.011844	C4b-binding protein alpha chain-like
15303201	2.2921	0.005042	B-cell CLL/lymphoma 3
15286684	2.2727	0.000212	amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase
15252920	2.2718	0.019833	probable G-protein coupled receptor 141-like
15332619	2.2582	0.000713	feline leukemia virus subgroup C cellular receptor 1
15297967	2.2421	0.003271	T-cell-interacting, activating receptor on myeloid cells protein 1-like
15280703	2.1977	0.000212	S100 calcium binding protein A11
15322702	2.1955	0.004586	tec protein tyrosine kinase
15190313	2.1901	0.024523	toll-like receptor 4
15223879	2.1739	0.0000675	scavenger receptor class B, member 1
15205901	2.1517	0.005746	M-phase phosphoprotein 8
15320209	2.1511	0.007568	RNA-binding protein 47-like
15187496	2.12	0.0000235	thioredoxin-related transmembrane protein 3
15253738	2.1063	0.002362	glutathione S-transferase P 1-like
15277372	2.0858	0.003524	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
15230062	2.0697	0.000753	scavenger receptor class B, member 1
15296514	2.0606	0.014064	free fatty acid receptor 2
15282065	2.0536	0.001214	rho GTPase-activating protein 29-like
15290462	2.05	0.005676	leukotriene A4 hydrolase
15297331	2.0208	0.003717	peptidoglycan recognition protein 1

Discussion

The porcine model for AL was a feasible model for the whole genome expression array study of whole blood using the Affymetrix porcine array. The mRNA in whole blood is derived mostly from lymphocytes (T-, B-, Natural Killer cells and monocytes). Circulating blood cells may carry valuable information in their RNA expression profile that may be indicative of incipient inflammatory processes (18). The Affymetrix microarray method was chosen. In doing so, financial expenditure was taken into account and

consideration was given to the possibility of obtaining a large number of mRNA assays, while at the same time being aware of the advantages of other methods commercially available (26). FC is a measure describing how much mRNA signal changes from an initial value (before rectal perforation) to a final value (4 hours after rectal perforation). It is defined as the difference between the initial to the final value. A positive FC indicates up-regulation of mRNA signal. In our study unlogged FC was used. Among the 19124 mRNA genes which significantly changed 4 hours after the perforation, 276 coded for an unknown protein and 48 coded for a known protein. The 11 up-regulated pathways with a high FC (over 4) in genes that coded for a known protein are known to be involved in increased inflammatory and immunological response, intracellular signaling and cell membrane regulation. All these findings are consistent with the pathophysiological process of acute inflammation/infection that occurs immediately after a rectal perforation. mRNA transcript with id 15242239 and corresponding protein tumor necrosis factor-alpha induced protein 6, was the protein with the highest FC, and is a secretory protein member of the hyaluronan-binding protein family. The hyaluronan-binding domain is known to be involved in extracellular matrix stability and cell migration. This gene can be induced by pro inflammatory cytokines such as TNF-α and interleukin-1 (27-29). Potassium channels are present in most mammalian cells, where they participate in a wide range of physiological responses. The protein encoded by this gene (id 15218761) is an integral membrane protein and inward-rectifier type potassium channel. The encoded protein has a greater tendency to allow potassium to flow into a cell rather than out of a cell (27-29). The third highest FC gene corresponds to the protein haptoglobin. This protein binds free plasma hemoglobin, allows degradative enzymes to gain access to the hemoglobin, preventing the loss of iron through the kidneys, thus protecting the kidneys from damage by hemoglobin. (27-29). Blood samples were analyzed 4 hours after a rectal perforation in pigs with the intention of emulating the reaction in the body immediately after an AL. The study concentrates on the 48 mRNAs encoding for a known protein and attempts to understand the initial pathophysiological processes immediately after the leakage. Ultimately, it would be beneficial to identify markers with high clinical sensitivity even during the first 3 days after an AL. Based on the results of this study, further studies should be conducted with an aim of finding appropriate clinical laboratory markers for AL.

Conclusions

Eleven known genes were consistently up-regulated after the rectal perforation. These genes were mainly involved in inflammatory response, intracellular signaling and cell membrane regulation. Their corresponding proteins could potentially be clinical biomarkers of anastomotic leakage and should be evaluated in further studies.

Limitations of the study

The porcine and human gastrointestinal tracts are very similar and, therefore, pigs have been used, however, antigens of specific proteins may vary significantly between the species. The study has only investigated mRNA that coded for known proteins, while the majority of m-RNA found in this study coded for an unknown protein. The study was only performed during the first 4 postoperative hours.

Abbreviations

Anastomotic leakage (AL)

C-reactive protein (CRP)

Intraperitoneal microdialysis (IPM)

Bioinformatics and Expression Analysis (BEA)

Fold Change (FC)

Messanger RNA (mRNA)

Declarations

Ethics approval and consent to participate: The experiment was approved by the Regional Animal Ethics Committee in Linköping (Dnr 174-3).

Consent to publish: Not applicable

Availability of data and materials: All data are available on request to the corresponding author

Authors' contributions:

Design of the study: Ioannis Oikonomakis, Kjell Jansson, David Brodin

Data Collection: Ioannis Oikonomakis, Tal M. Hörer, Per Skoog, Jenny Seilitz, Kristofer F. Nilsson

mRNA analysis and statistics: David Brodin

Analysis of data and manuscript: Ioannis Oikonomakis, David Brodin, Tal M. Hörer, Per Skoog, Jenny Seilitz, Kristofer F. Nilsson, Adrian D. Meehan, Kjell Jansson

References

1. Matthiessen P, Hallbook O, Rutegard J, Simert G, Sjodahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Annals of surgery. 2007;246(2):207-14.
2. Daams F, Wu Z, Lahaye MJ, Jeekel J, Lange JF. Prediction and diagnosis of colorectal anastomotic leakage: A systematic review of literature. World journal of gastrointestinal surgery. 2014;6(2):14-26.
3. Matthiessen P, Strand I, Jansson K, Tornquist C, Andersson M, Rutegard J, et al. Is early detection of anastomotic leakage possible by intraperitoneal microdialysis and intraperitoneal cytokines after anterior resection of the rectum for cancer? Diseases of the colon and rectum. 2007;50(11):1918-27.

4. Reynolds IS, Boland MR, Reilly F, Deasy A, Majeed MH, Deasy J, et al. C-reactive protein as a predictor of anastomotic leak in the first week after anterior resection for rectal cancer. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland. 2017;19(9):812-8.
5. Kjell Jansson TJ, Professor Lars Norgren. Intraperitoneal microdialysis: a new method to monitor patients after abdominal surgery. International journal of intensive care. 2003;10(spring 2003, Number 1).
6. Jansson K, Ungerstedt J, Jonsson T, Redler B, Andersson M, Ungerstedt U, et al. Human intraperitoneal microdialysis: increased lactate/pyruvate ratio suggests early visceral ischaemia. A pilot study. Scandinavian journal of gastroenterology. 2003;38(9):1007-11.
7. Ellebaek M, Qvist N, Fristrup C, Mortensen MB. Mediastinal microdialysis in the diagnosis of early anastomotic leakage after resection for cancer of the esophagus and gastroesophageal junction. American journal of surgery. 2014;208(3):397-405.
8. Ellebaek Pedersen M, Qvist N, Bisgaard C, Kelly U, Bernhard A, Moller Pedersen S. Peritoneal microdialysis. Early diagnosis of anastomotic leakage after low anterior resection for rectosigmoid cancer. Scand J Surg. 2009;98(3):148-54.
9. Jansson DT, Oikonomakis I, Strand IEU, Meehan A, Jansson K. Metabolism, inflammation and postoperative time are the key to eraly diagnosis of anastomotic leak. Journal of Surgery and Surgical Research. 2019;5(2):078-85.
10. Oikonomakis I, Jansson D, Hörer TM, Skoog P, Nilsson KF, Jansson K. Results of postoperative microdialysis intraperitoneal and at the anastomosis in patients developing anastomotic leakage after rectal cancer surgery. Scandinavian journal of gastroenterology. 2019;54(10):1261-8.
11. Jansson K, Jansson M, Andersson M, Magnuson A, Ungerstedt U, Norgren L. Normal values and differences between intraperitoneal and subcutaneous microdialysis in patients after non-complicated gastrointestinal surgery. Scandinavian journal of clinical and laboratory investigation. 2005;65(4):273-81.
12. van Berge Henegouwen MI, van der Poll T, van Deventer SJ, Gouma DJ. Peritoneal cytokine release after elective gastrointestinal surgery and postoperative complications. American journal of surgery. 1998;175(4):311-6.
13. Jansson K, Redler B, Truedsson L, Magnuson A, Ungerstedt U, Norgren L. Postoperative on-line monitoring with intraperitoneal microdialysis is a sensitive clinical method for measuring increased anaerobic metabolism that correlates to the cytokine response. Scandinavian journal of gastroenterology. 2004;39(5):434-9.
14. Tamion F, Richard V, Lyoumi S, Daveau M, Bonmarchand G, Leroy J, et al. Gut ischemia and mesenteric synthesis of inflammatory cytokines after hemorrhagic or endotoxic shock. The American journal of physiology. 1997;273(2 Pt 1):G314-21.
15. Sammour T, Singh PP, Zargar-Shoshtari K, Su'a B, Hill AG. Peritoneal Cytokine Levels Can Predict Anastomotic Leak on the First Postoperative Day. Diseases of the colon and rectum. 2016;59(6):551-6.

16. Sparreboom CL, Wu Z, Dereci A, Boersema GS, Menon AG, Ji J, et al. Cytokines as Early Markers of Colorectal Anastomotic Leakage: A Systematic Review and Meta-Analysis. Gastroenterology research and practice. 2016;2016:3786418.
17. Yamamoto T, Umegae S, Matsumoto K, Saniabadi AR. Peritoneal cytokines as early markers of peritonitis following surgery for colorectal carcinoma: a prospective study. Cytokine. 2011;53(2):239-42.
18. Rissland OS. The organization and regulation of mRNA-protein complexes. Wiley Interdiscip Rev RNA. 2017;8(1).
19. Kurahashi I, Fujita Y, Arao T, Kurata T, Koh Y, Sakai K, et al. A microarray-based gene expression analysis to identify diagnostic biomarkers for unknown primary cancer. PLoS One. 2013;8(5):e63249.
20. Kerns RT, Miles MF. Microarray analysis of ethanol-induced changes in gene expression. Methods Mol Biol. 2008;447:395-410.
21. Block T, Isaksson HS, Acosta S, Björck M, Brodin D, Nilsson TK. Altered mRNA expression due to acute mesenteric ischaemia in a porcine model. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2011;41(2):281-7.
22. Chawla LS, Toma I, Davison D, Vaziri K, Lee J, Lucas R, et al. Acute appendicitis: transcript profiling of blood identifies promising biomarkers and potential underlying processes. BMC Med Genomics. 2016;9(1):40.
23. Rai MF, Sandell LJ, Zhang B, Wright RW, Brophy RH. RNA Microarray Analysis of Macroscopically Normal Articular Cartilage from Knees Undergoing Partial Medial Meniscectomy: Potential Prediction of the Risk for Developing Osteoarthritis. PLoS One. 2016;11(5):e0155373.
24. Keen HL, Sigmund CD. Microarray Analysis of Hypertension. Methods Mol Biol. 2017;1527:41-52.
25. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010;8(6):e1000412.
26. Meyer A, Paroni F, Günther K, Dharmadhikari G, Ahrens W, Kelm S, et al. Evaluation of Existing Methods for Human Blood mRNA Isolation and Analysis for Large Studies. PLoS One. 2016;11(8):e0161778.
27. Alekseyev YO, Fazeli R, Yang S, Basran R, Maher T, Miller NS, et al. A Next-Generation Sequencing Primer-How Does It Work and What Can It Do? Acad Pathol. 2018;5:2374289518766521.
28. Harris MA, Clark J, Ireland A, Lomax J, Ashburner M, Foulger R, et al. The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res. 2004;32(Database issue):D258-61.
29. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019;47(D1):D506-d15.

Figures

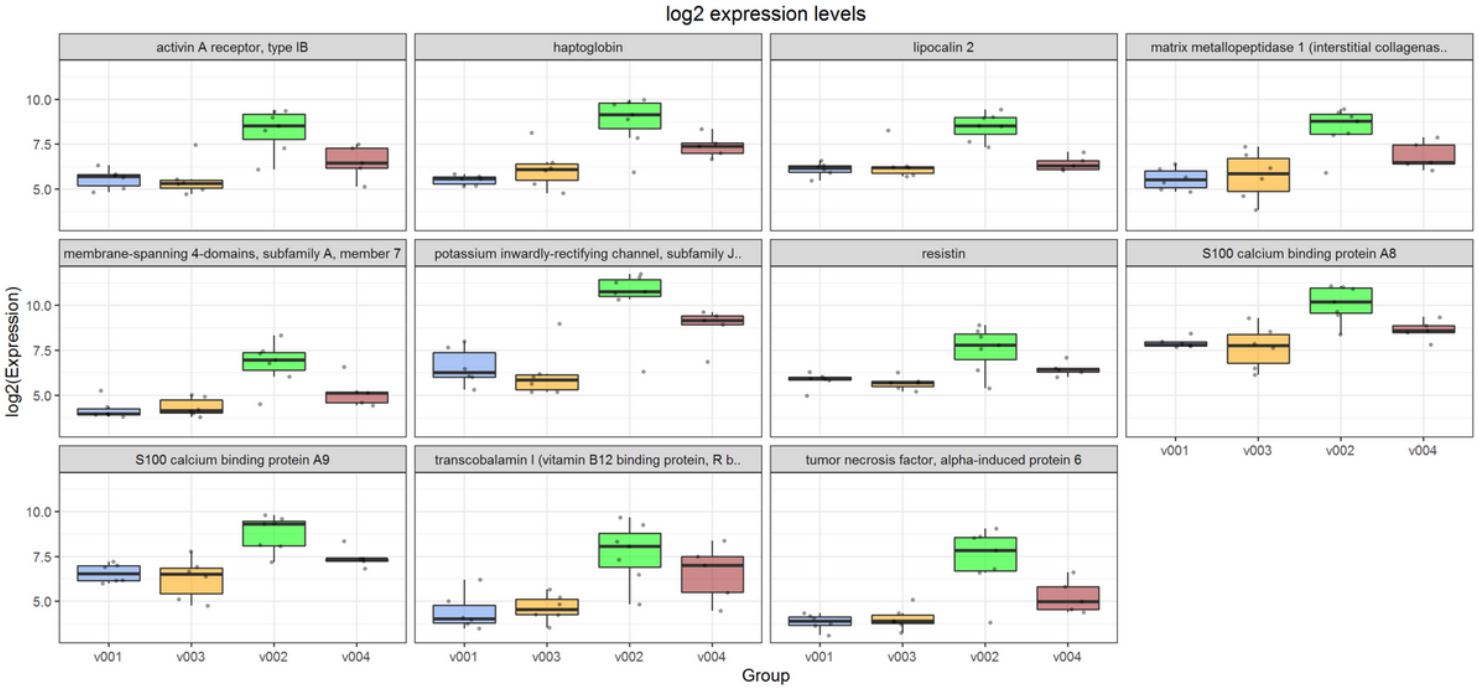

Figure 1

Boxplot- 11 shows the known genes with FC> 4. Preoperative protein expression levels in the experimental group shown in blue, preoperative protein expression levels in the control group shown in yellow, postoperative protein expression levels shown in green in the experimental group and in red the control group. The black line in the boxes shows median value. For a better overview, FC values in the y-axis have been logarithmized. All statistical calculations are performed on non-logarithmic values.

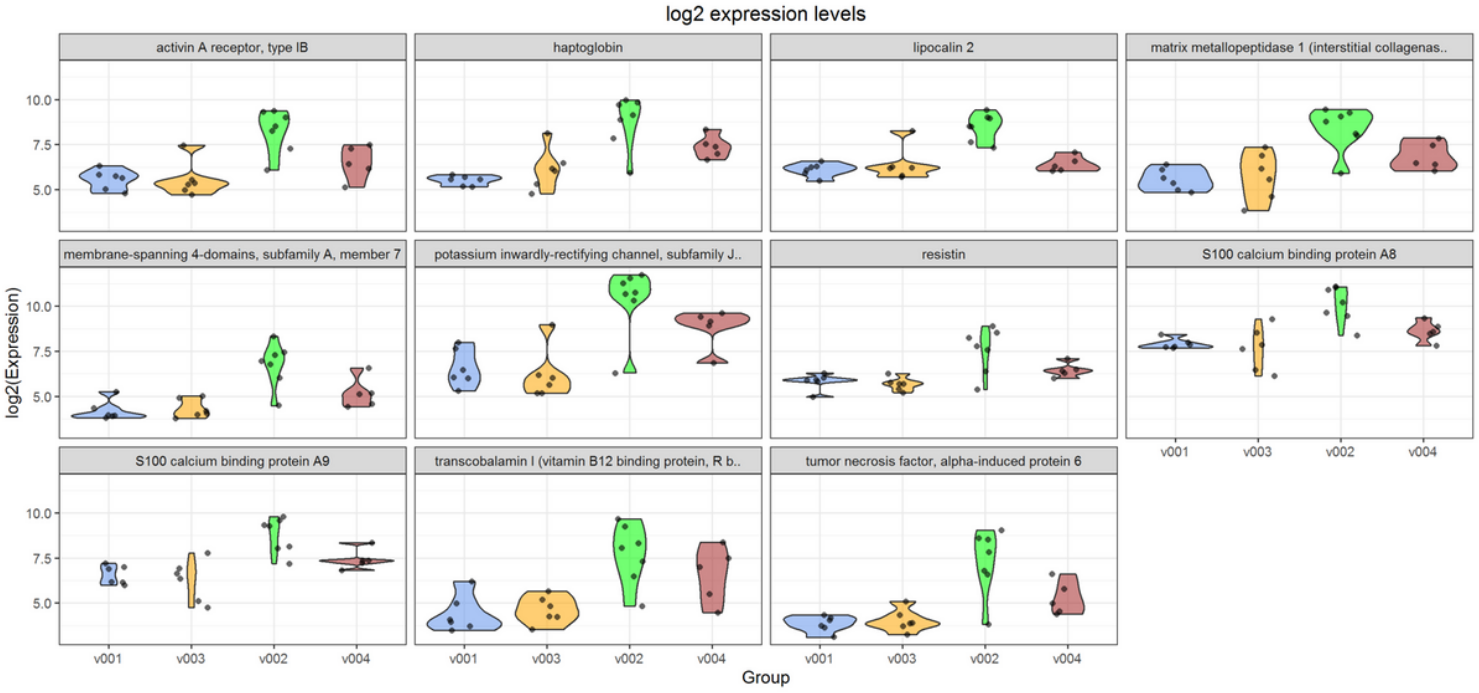

Figure 2

Violin plot-11 shown known genes with FC>4. Preoperative protein expression levels in the experimental group shown in blue, preoperative protein expression levels in the control group shown in yellow, postoperative protein expression levels shown in green in the experimental group and in red the control group. For a better overview, FC values in the y-axis have been logarithmized. All statistical calculations are performed on non-logarithmic values.

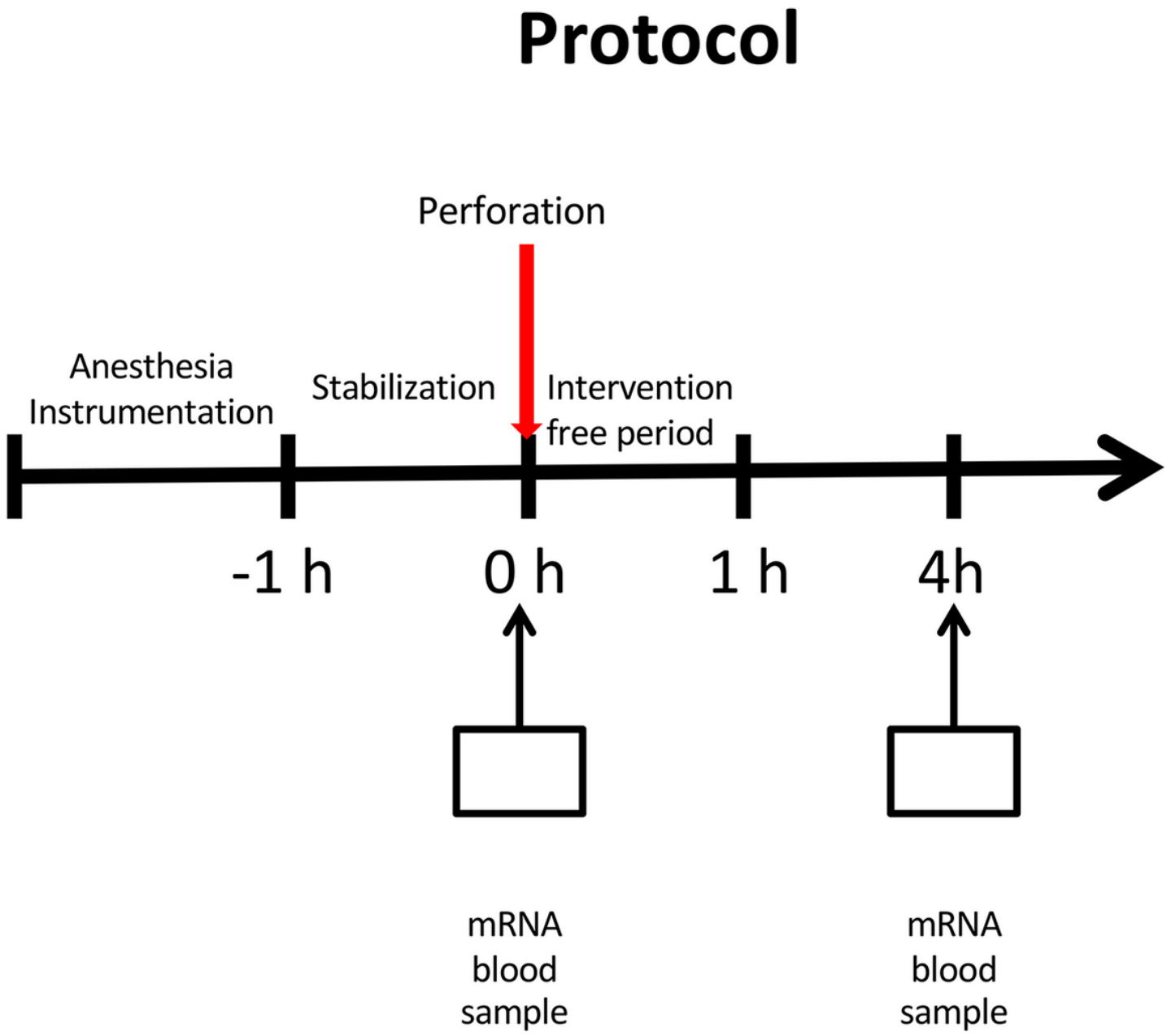

Figure 3

Experiment protocol