Maria Dung Cao

MR metabolic characterization of locally advanced breast cancer

– treatment effects and prognosis

Thesis for the degree of Philosophiae Doctor

Trondheim, March 2012

Norwegian University of Science and Technology
Faculty of Medicine
Department of Circulation and Medical Imaging

NTNU – Trondheim
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the degree of Philosophiae Doctor

Faculty of Medicine
Department of Circulation and Medical Imaging

ISBN 978-82-471-3420-7 (printed ver.)
ISBN 978-82-471-3421-4 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2012:74

Printed by NTNU-trykk

Sammendrag

Brystkreft er den hyppigste kreftsykdommen blant kvinner. Lokalavansert brystkreft utgjør omtrent 10% av alle brystkrefttilfeller og omfatter en heterogen pasientgruppe med ulike prognoser. Pasienter med lokalavansert brystkreft får ofte kjemoterapi før kirurgisk fjerning av tumor, såkalt neoadjuvant kjemoterapi, for å redusere størrelsen på tumoren. Det er stor variasjon i behandlingsrespons for denne pasientgruppen, og det er derfor behov for å utvikle målrettet og individualisert behandling, samt metoder for oppfølging av behandlingsrespons.

Metabolomics er en systematisk analyse av småmolekylære forbindelser (metabolitter) i biologiske prøver. Den metabolske profilen til brystkreftvev er vist å korrelere med viktige kliniske parametere, for eksempel tumorgrad, hormonreseptorstatus og lymfeknutespredning. Magnetisk resonans (MR) spektroskopi er en metode som kan gi en detaljert beskrivelse av metabolittprofilen i vevet. En fordel med denne metoden er at vevsprøven er intakt etter analysen slik at den samme vevsprøven kan analyseres videre med andre metoder, for eksempel immunohistokjemi, genuttrykk- eller proteinanalyse.

En sentral gruppe metabolitter innenfor brystkreftforskning er kolinforbindelser. Disse metabolittene er viktig for celledeling, signaloverføring, lipidmetabolisme og cellens membranstruktur. Laktat er en annen viktig metabolitt som inngår i energimetabolismen. I dette arbeidet ble vevsprøver fra pasienter med lokalavansert brystkreft analysert ved bruk av MR spektroskopi for prediksjon av behandlingsrespons og overlevelse. I tillegg undersøkte vi rollen til glycerophosphodiester phosphodiesterase (GDPD) i regulering av kolinforbindelser.

Sammendrag

Alle pasienter hadde en effekt av behandlingen som ble gitt, og nesten alle fikk en reduksjon i tumorstørrelse etter behandling. Resultatene viste ingen metabolske forskjeller mellom pasienter med klinisk god eller dårlig behandlingsrespons. Resultatene viser derimot at de metabolske forandringene som skjer under neoadjuvant kjemoterapi er forskjellig i pasienter som overlever mer enn fem år og de som dør før fem år. Høyere nivå av laktat, glycine og kolinforbindelser etter behandling var forbundet med dårlig prognose. Analysene av GDPD5 tyder på at enzymet er involvert i reguleringen av kolinmetabolismen, men dets rolle for bruk i målrettet terapi er fortsatt uklart og nye studier må til for å undersøke dette.

MR metabolomics kan brukes til å undersøke metabolske forandringer under neoadjuvant kjemoterapi behandling og kan identifisere viktige metabolitter for prediksjon av overlevelse hos pasienter med lokalavansert brystkreft.

Kandidat: Maria Dung Cao

Institutt: Institutt for Sirkulasjon og bildediagnostikk

Veileder(e): Ingrid S. Gribbestad, Beathe Sitter, Tone F. Bathen og Steinar Lundgren

Finansieringskilde: Norges forskningsråd, FRIMED programmet

Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden Philosophiae Doctor i molekylær medisin.
Disputas finner sted i Auditoriet, Medisinsk teknisk forskningssenter, onsdag 14.mars 2012, kl12.15.

Acknowledgement

The work presented in this thesis has been carried out at the MR Center, Department of Circulation and Medical Imaging, NTNU, in the period August 2008 – December 2011. Financial support was provided by the Research Council of Norway, through the FRIMED program.

I sincerely appreciate the collaboration and support from many people that have contributed to the completion of this thesis. In particular I want to express my gratitude to my main supervisor, Professor Ingrid S. Gribbestad, and my co-supervisors, Dr. Beathe Sitter, Dr. Tone F. Bathen, and Professor Steinar Lundgren for your professional guidance and valuable advices. Ingrid, for your enthusiasm and insight. Your knowledge and encouragement have inspired me to pursue my interest in research. Tone, for your help and expertise in statistical data analyses. Beathe, for sharing your extensive knowledge in HR MAS MRS. Steinar, for being so helpful and always providing me the data I needed. I would also like to thank my co-authors for their contributions, especially Professor Per E. Lønning for providing the patient samples and Assoc. Prof Anna Bofin for introducing me to histopathology. Special thanks to Guro F. Giskeødegård for the scientific discussions and support as a colleague and friend. And to all my colleagues, thank you for providing such a positive and nice working environment. I especially appreciate the help and support from Dr. Torill E. Sjøbakk. Thank you for always be there when I needed your help.

I would like to thank Dr. Kristine Glunde for given me the opportunity for a research stay at Johns Hopkins University School of Medicine (JHU) in Baltimore, USA. Thank you for your guidance and productive discussions. I would also like to thank the

researches and students at the *In vivo* Cellular and Molecular Imaging Center (JHU) for welcoming me to your group. I had a very nice stay and learned a lot.

Special thanks to the women participating in the research studies. This work would not have been possible without you.

On a more personal level I am especially grateful for my family and friends, who have always been there for me. Special thanks goes to my parents, Dang and Nhung, my brothers and sisters, Nhat, Son, Thu, and Thao, and my close friends. Thank you for your endless support and for believing in me.

Maria Dang Cao

Trondheim, December 2011

Summary

Breast cancer is the most frequent cancer disease among women globally. Locally advanced breast cancer (LABC) constitutes a heterogeneous group of patients with variable prognosis. Today's treatment decision is predominately based on clinical assessment, histopathological evaluation, and hormone receptor and lymph node status. So far, these data are not sufficient for designing a proper personalized treatment or accurately predicting treatment response and survival. Molecular characterization of tumors may help stratifying patients for individualized treatment, thereby achieving better prognosis.

Magnetic resonance (MR) metabolomics analyses assess the downstream products of gene and protein expressions, i.e. the metabolites, and have shown to provide both predictive and prognostic information for several types of cancers. Proton high resolution magic angle spinning (^{1}H HR MAS) MR spectroscopy is a non-destructive and high-throughput technique that provides highly resolved MR spectra from biological tissue. Recently, altered cell metabolism is suggested as a new emerging hallmark of cancer. Choline phospholipid metabolism is involved in cell signaling, lipid metabolism, and the structural integrity of the cell membrane. Several MRS studies have suggested the total choline-containing metabolite (tCho) level as an *in vivo* biomarker for diagnosis and treatment evaluation of breast cancer. Reprogramming of energy metabolism and activation of tumor hypoxic response are commonly observed in cancers, and can be characterized by high lactate production.

In this thesis, multivariate data analyses and metabolite quantification of ^{1}H HR MAS MRS data were performed to investigate the potential of metabolomics for prediction of clinical response and long-term survival in LABC patients receiving neoadjuvant

chemotherapy (NAC). In addition, the role of glycerophosphodiester phosphodiesterase (GDPD) in choline phospholipid metabolism of human breast cancer was investigated.

All patients had a metabolic response to NAC and almost all patients had a reduction in tumor size. Our results show no clear differences in metabolic responses to NAC between patients with partial response and stable disease and no significant multivariate models for prediction of clinical response by MR metabolomics data. In general, all patients experienced a decrease in tCho levels. It is possible that a cohort including also patients with progressive disease would reveal clearer differences in the metabolite profiles between the clinical response groups. This thesis demonstrates that MR metabolomics contain prognostic information that is associated with survival status of LABC patients. Increase in lactate levels as a response to NAC was associated with low survival rates (< 5 years), while decreased glycine and choline phospholipid metabolites were associated with long-term survival (≥ 5 years). The observed metabolite profiles consisting of higher levels of lactate, glycine, and tCho post-treatment were predictive of low breast cancer survival rates.

GDPD5 gene expression was correlated with choline phospholipid metabolite levels and with CHKA and PLD1 gene expressions suggesting GDPD5 to have a role in regulation of choline phospholipid metabolism in human breast cancer. However, more studies are needed to investigate the relationship between GDPD5 and tumor malignancy, and also estrogen receptor status, for use as target in breast cancer treatment.

In conclusion, monitoring metabolic responses to NAC by MR metabolomics may have the potential to assist the prediction of survival and help identify new targets for therapeutic treatment of breast cancer.

Symbols and abbreviations

μ	magnetic moment
^{1}H HR MAS	proton high-resolution magic angle spinning
AJCC	American Joint Committee on Cancer
AQ	acquisition time
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
B_0	external magnetic field
BRCA	breast cancer susceptibility gene
CDP-choline	cytidine diphosphate choline
CHKA	choline kinase alpha
Cho	free choline
CHT-1	choline transporter-1
CPMG	Carr-Purcell-Meiboom-Gill
CR	complete response
CT	computerized tomography
ct	threshold cycle
CTP	phosphocholine cytidylyltransferase
CV	cross-validation
EGFR	epidermal growth factor receptor
ER	estrogen receptor
ERETIC	electronic reference to access in vivo concentration
FBS	fetal bovine serum
FEC	5-flourouracil, epirubicin and cyclophosphamide
FID	free-induction decay
GA	genetic algorithm
GDPD5	glycerophosphodiester phosphodiesterase domain containing 5
GPC	glycerophosphocholine
GPC-PDE	glycerophosphocholine phosphodiesterase
HER2	human epidermal growth factor receptor 2
HES	hematoxylin-eosin-saffron
HiF-1	hypoxia inducible factor-1
HR	high-resolution
HRP	horseradish peroxidase

I	spin quantum number
IBC	inflammatory breast cancer
IDC	invasive ductal carcinoma
ILC	invasive lobular carcinoma
LABC	locally advanced breast cancer
LDH	lactate dehydrogenase
LV	latent variable
LYSO-PLA1	lysophospholipase A1
MAS	magic angle spinning
MCT	monocarboxylate transporters
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAC	neoadjuvant chemotherapy
NBCG	Norwegian Breast Cancer Group
NPP6	nucleotide pyrophosphatases/phosphodiesterases 6
OCT2	organic cation transporter-2
PBS	phosphate buffered saline
PC	phosphocholine
PC	principal component
PCA	principal component analysis
pCR	pathological complete response
PD	progressive disease
PgR	progesterone receptor
PLA2	phospholipase A2
PLC	PtdCho-specific phospholipase C
PLD	PtdCho-specific phospholipase D
PLS-DA	partial least squares discriminant analysis
ppm	parts-per million
PR	partial response
PtdCho	phosphatidylcholine
PULCON	pulse length based concentration determination
pw	pulse width
qRT-PCR	quantitative real-time PCR
RECIST	Response Evaluation Criteria on Solid Tumors
RF	radio frequency
RIN	RNA integrity number
ROC	receiver operating characteristic
S/N	signal-to-noise ratio
SD	stable disease
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
T_1	longitudinal relaxation time
T_2	transversal relaxation time

tCho	choline-containing metabolites
TE	echo time
TMS	tetramethylsilane
TNM	extend of tumor (T), degree of spread to lymph nodes (N), distant metastasis (M)
TR	repetition time
TSP	trimethylsilyl propionic acid
UICC	Union for International Cancer Control
VEGF	vascular endothelial growth factor
VIP	variable importance in the projection
WHO	World Health Organization
ω_r	spin-rate

List of papers

Paper I

Predicting long-term survival and treatment response in breast cancer patients receiving neoadjuvant chemotherapy by MR metabolic profiling

Maria D. Cao, Beathe Sitter, Tone F. Bathen, Anna Bofin, Per E. Lønning, Steinar Lundgren and Ingrid S. Gribbestad

NMR Biomed. 2012 Feb;25(2):369-78.

Paper II

Prognostic value of metabolic response in breast cancer patients receiving neoadjuvant chemotherapy

Maria D. Cao*, Guro F. Giskeødegård*, Tone F. Bathen, Beathe Sitter, Anna Bofin, Per E. Lønning, Steinar Lundgren and Ingrid S. Gribbestad (*shared first authorship)

Revised version accepted. *BMC Cancer*. 2012 Jan 25;12(1):39.

Paper III

Glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) expression correlates with malignant choline phospholipid metabolite profiles in human breast cancer

Maria D. Cao, Mailin Doepkens, Balaji Krishnamachary, Farhad Vesuna, Mayur M. Gadiya, Per E. Lønning, Zaver M. Bhujwalla, Ingrid S. Gribbestad and Kristine Glunde

NMR Biomed. 2012 Jan 26. doi: 10.1002/nbm.2766.

Contents

1 Introduction

1.1 Breast cancer

Breast cancer is the most frequent cancer disease among women globally with approximately 1.6 million new cases diagnosed in 2010 [1]. Despite improvements in early detection and treatment, breast cancer is still one of the leading causes of cancer-related death among women globally with an estimated 425 000 deaths in 2010 [1]. In Norway, 2745 newly diagnosed breast cancer cases and 671 breast cancer deaths were reported for women in 2009 [2]. The incidence of breast cancer is higher in the western countries than in developing countries, but the mortality rate is higher in less developed regions of the world [3]. These differences can in part be explained by different access to medical facilities and treatment strategies.

Factors associated with breast cancer risk can be genetic or non-genetic. Hereditary breast cancer accounts for 5-10% of all breast cancer cases and is mostly caused by germline mutations in the two first identified breast cancer susceptibility genes, BRCA1 and BRCA2 [4]. Exposure to reproductive hormones, such as estrogen and progesterone, are thought to increase breast cancer risk through their influence on cell proliferation [5]. Many non-genetic factors associated with increased breast cancer risk have been identified including age, early menarche and late menopause, late age at first childbirth (> 35 year), no breastfeeding, use of oral contraceptives and post-menopausal hormones, post-menopausal obesity, alcohol consumption, and physical inactivity [6, 7].

The female breast consists of lobules (made of lobular epithelial cells that line the milk-producing glands), ducts (made of duct epithelial cells that line the tubes conveying milk secretion), fatty tissue, and connective tissue. Carcinoma originating from epithelial cells is the most common type of breast cancer [8]. During cancer transformation normal epithelial cells lining the ducts or lobules are replaced by malignant atypical epithelial cells (Figure 1.1). Invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) account for approximately 80% and 10-20% of breast cancer cases, respectively [8].

The overall 5-year breast cancer survival rate is approximately 80% for all patients, but is lower for advanced breast cancer [9]. Locally advanced breast cancer (LABC) constitutes a heterogeneous group of patients with variable prognosis and with a 5-year survival rate of 50-80% [10]. LABC can be defined as the most advanced stage before metastatic disease and constitutes approximately 10% of the newly diagnosed breast cancers [11]. The incidence of LABC is higher in developing countries than in western countries probably due to the occurrence of late-stage disease at the time of diagnosis [11].

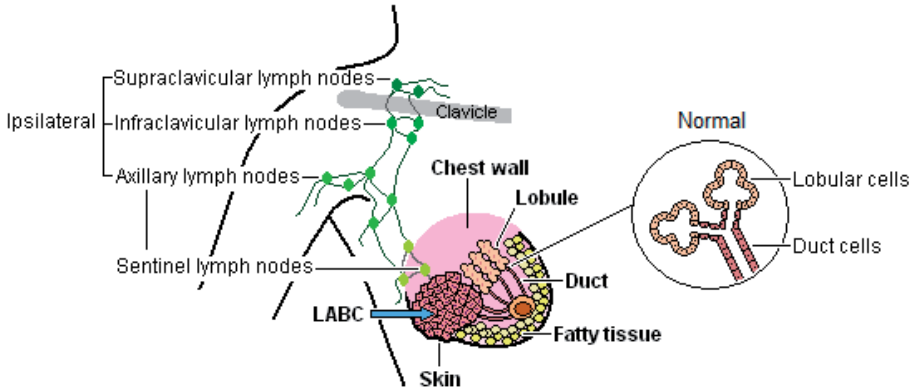

Figure 1.1: Anatomical illustration of locally advanced breast cancer (LABC). LABC can originate from ductal or lobular epithelial cells. The tumors are either > 5 cm or of any size with extension to chest wall, skin or with lymph node metastasis. The different types of metastatic lymph nodes are illustrated, see Table 1.1.

1.1.1 Staging of breast cancer

Breast cancer staging can be defined using the TNM classification system which takes into consideration the extent of the tumor (T), the degree of spread to lymph nodes (N), and distant metastasis (M) (Table 1.1) [12]. The TNM system is used to describe the extent of the disease and is indeed an important prognostic factor for determining treatment options and survival probabilities. The TNM clinical categories are determined by physical examinations and imaging modalities such as mammography and ultrasound of the breast, and often bone scintigram, and computerized tomography (CT) examination of lung and liver. Based on the TNM system, the patients are divided into different breast cancer stages ranging from 0 to IV. Stage 0 is used to describe non-invasive breast cancer, while stages I to III include invasive breast cancer with or without lymph node metastasis. Stage IV describes invasive breast cancer that has spread to other organs [12].

There is no international standard for the staging of LABC. In general, LABC can be defined as T3-T4 (any N) or N2-N3 (any T) without distant metastasis (M0), which includes all patients of stage IIIA-C disease and a subset of stage IIB. According to the TNM system, inflammatory breast cancer (IBC) is considered a subgroup of LABC [12]. However, IBC is often discussed separately due to its distinct clinical presentation and poor prognosis compared to non-IBC [13].

Table 1.1 TNM clinical classification according to the AJCC/UICC [12]

	Characteristic	
T0	No evidence of primary tumor	
T1	Tumor ≤ 2 cm in greatest dimension	
T2	Tumor > 2 cm, but ≤ 5 cm in greatest dimension	
T3	Tumor > 5 cm in greatest dimension	LABC
T4	Tumor of any size with extension to chest wall or skin or inflammatory carcinoma	LABC
N0	No regional lymph node metastasis	
N1	Metastasis in movable ipsilateral axillary lymph node(s)	
N2	Metastasis in fixed ipsilateral axillary lymph node(s)	LABC
N3	Metastasis in ipsilateral infraclavicular or supraclavicular lymph node(s)	LABC
M0	No distant metastasis	
M1	Distant metastasis	

TNM categories associated with locally advanced breast cancer (LABC) are highlighted. AJCC, American Joint Committee on Cancer; UICC, Union for International Cancer Control.

1.1.2 Prognostic and predictive factors

Prognostic factors can predict the outcome of a disease, while predictive factors can predict the response to a specific treatment. The prognostic factors for LABC are similar to the prognostic factors for earlier stage breast cancer with tumor size, grade, and lymph node status being the strongest prognostic factors for breast cancer survival [14].

Estrogen receptor (ER) and progesterone receptor (PgR) are ligand-dependent nuclear transcription factors involved in proliferative activity and reproductive development. Overexpression of ER and PgR is typically observed in breast cancer and is thought to play a major role in breast cancer progression [5]. The mechanisms behind the abnormal expression of these reproductive hormone receptors are still under investigation. Interestingly, both germline and somatic mutations in the ER gene of breast carcinomas are in fact rare [15]. Patients with ER and PgR positive tumors have better prognosis compared to patients that are negative for these hormone receptors [16].

Human epidermal growth factor receptor-2 (HER2, HER-2/neu, c-erbB2) is a relatively newly discovered biological target for the treatment of breast cancer. HER2 is a member of the epidermal growth factor receptor (EGFR) family which regulates several signal transduction pathways of cell growth and differentiation. The expression of HER2 is increased in approximately 20% of breast carcinomas and is associated with increased proliferation, high metastatic potential and poor outcome [17, 18].

Hormone receptor status and HER2 expression are used as target for breast cancer treatment. Thus, they can be defined as both prognostic and predictive factors. The change in tumor size in response to treatment is an important predictive factor for treatment evaluation. In the last decade, molecular characterization by metabolic profiling has provided potential new factors that may play an important role in diagnosis, treatment evaluation, and prognosis of breast cancer [19].

1.2 Treatment strategies for LABC

The treatment regimens recommended for LABC patients vary between countries. In general, the patients are often treated with chemotherapy before surgery, so-called neoadjuvant chemotherapy (NAC), to shrink the tumor enough to make surgical removal possible and even allow for breast conserving surgery in exchange of a mastectomy. After surgery, post-operative treatment is usually given in the same way as for those with earlier stage breast cancer. In Norway, guidelines for treatment regimen of LABC are given by the Norwegian Breast Cancer Group (www.NBCG.no).

1.2.1 Neoadjuvant chemotherapy (NAC)

Primary systemic treatment (or NAC) was first described in the 1970's [20] and is now well established in the treatment of LABC [10]. For LABC patients, treatment with NAC is given to make a tumor operable, either by mastectomy or breast-conserving surgery. Overall, NAC offers direct evaluation of treatment effects and the response to NAC has been shown to be an important predictor of survival [21, 22]. Better clinical and pathological response to NAC is associated with prolonged recurrence-free survival [21, 22]. In Norway, the current standard treatment for LABC is a combination of the cytostatic agents 5-flourouracil, epirubicin, and cyclophosphamide (FEC) and often sequential treatment of FEC and a taxane substance (www.NBCG.no).

Anthracyclines were discovered in the 1960's and include doxorubicin and epirubicin which are among the most effective chemotherapeutic agents for the treatment of breast cancer. These agents predominately accumulate in neoplastic and proliferating cells where they are capable of inducing DNA strand breakage and apoptosis through their interaction with DNA binding proteins, tumor suppressor gene p53, and DNA intercalation [23-25]. Another relatively new recommended drug group developed in the 1990's for use in the treatment of breast cancer is taxane. Paclitaxel is a taxane drug that causes mitotic arrest by disturbing the breaking and rearranging of microtubules in the cells [26].

1.2.2 Post-operative treatment

Post-operative treatment such as radiation, hormone, and biologic therapies are usually given to LABC patients. For women at high risk of recurrence, like LABC patients, the breast cancer survival rate has been shown to significantly improve when treated with radiation therapy [27]. Patients with ER positive tumors could benefit from anti-estrogen drugs, such as tamoxifen, which can result in a reduction of recurrences and breast cancer death [28, 29]. Treatment with trastuzumab, a monoclonal antibody against HER2, can reduce the recurrence rate and mortality of breast cancer [18].

1.2.3 Clinical response evaluation

The change in tumor size can be used as a predictive factor of a specific treatment. The tumor response criteria were first published by the World Health Organization (WHO) in 1981 (Table 1.2) [30]. In 2000, an improved and standardized response evaluation system was introduced, known as Response Evaluation Criteria on Solid Tumors (RECIST) (Table 1.2) [31]. Imaging modalities such as mammography, ultrasound, and magnetic resonance imaging (MRI) can be used for the evaluation of treatment response. MRI has been shown to correlate the best with pathological findings compared to clinical examination, mammography, and ultrasound [32]. However, in a neoadjuvant setting when the breast lesions are large (> 5 cm in greatest dimension), the response to treatment can be evaluated clinically using a caliper [31].

Table 1.2 Clinical response criteria for target lesions according to WHO and RECIST criteria [30, 31]

	WHO*	**RECIST**
	Bidimensional **Product of the two largest diameters**	Unidimensional **The longest diameter**
Complete response (CR)	Disappearance of all target lesions	Disappearance of all target lesions
Partial response (PR)	≥ 50% decrease	≥ 30% decrease
Stable disease (SD)	< 50% decrease to < 25% increase	< 30% decrease to < 20% increase
Progressive disease (PD)	≥ 25% increase	≥ 20% increase

* Clinical response criteria used in paper I and II.

1.3 The biology of cancer

Carcinogenesis is a multistep process that involves essential genomic alternations that collectively dictate malignant growth. Six hallmarks of cancer have been suggested as novel characteristics of cancer cells, including being self-sufficient in growth signals, insensitive to anti-growth signals, limitless replicative potential, evading apoptosis, sustained angiogenesis, and tissue invasion [33]. Two emerging physiological features, reprogramming of energy metabolism and evading immune destruction, have recently been added to the list [34].

1.3.1 Tumor glycolysis

Cancer cells have the ability to adjust their energy metabolism in order to promote rapid and uncontrolled cell proliferation. Glycolysis is a metabolic process in which glucose is converted into pyruvate to produce energy in the form of adenosine triphosphate (ATP). The total number of ATP per glucose molecule depends on the metabolic fate of pyruvate, which in normal cells depend on oxygen availability. In non-proliferating cells under condition of sufficient oxygen supply, pyruvate is completely oxidized in the mitochondria through oxidative phosphorylation which results in high ATP production (Figure 1.2). During anaerobic glycolysis, only a minimum number of ATP is produced by converting pyruvate to lactate. In cancer cells, most pyruvate is converted to lactate regardless of the oxygen levels, so-called aerobic glycolysis. This glycolytic switch in cancer cells was first described by Warburg in the 1950's [35].

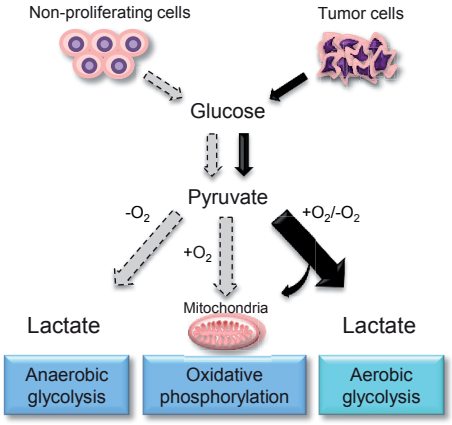

Figure 1.2: Energy metabolism in non-proliferating and tumor cells. In case of low oxygen supply, normal cells can undergo anaerobic glycolysis and generate lactate instead of mitochondrial oxidative phosphorylation to allow for glycolysis to continue. Tumor cells tend to convert most glucose to lactate regardless of the oxygen supply. This reprogramming of energy metabolism appears to promote tumor growth.

It is not fully understood why cancer cells prefer the lower efficiency of ATP production than mitochondrial oxidative phosphorylation. It has been suggested that aerobic glycolysis protects cancer cells from acid-induced cell toxicity and is an adaption to intermittent hypoxia in pre-malignant lesions [36]. In addition, increased glycolysis has been shown to facilitate the uptake and incorporation of nutrients needed for cell proliferation [37]. Also, glycolytic intermediates are involved in various biosynthetic pathways that facilitate cell proliferation [37, 38]. The metabolite glycine is an amino acid involved in the nucleotide and protein synthesis, and is derived from the glycolytic intermediate 3-phosphoglycerate, but can also be synthesized from choline through the glycine-betaine pathway. The levels of glycine and lactate have been associated with malignancy and prognosis in different types of cancers [39-45]. However, the molecular mechanisms behind the influence of glycine and lactate on malignant behavior of cancer cells are not fully understood.

1.3.2 Tumor hypoxia and angiogenesis

Normal cells have the capacity to adapt to conditions of low oxygen supply by increasing their oxygenation through the activation of hypoxia inducible factor-1 (HiF-1) transcription factor. HiF-1 regulates multiple downstream genes involved in glucose transportation, the glycolytic pathway, oxygen capacity, and angiogenesis (e.g. vascular endothelial growth factor, VEGF) [46-49]. Hypoxia is typically observed in solid tumors as a result of poor blood supply due to either large tumor size or chaotic and dysfunctional vasculature. Tumor cells appear to have taken advantage of the hypoxic response system to facilitate cell proliferation and angiogenesis which are required for tumor growth and metastasis [50]. Furthermore, HIF-1 plays a regulative role in the production and transportation of lactate by influencing the expression of lactate dehydrogenase (LDH) and monocarboxylate transporters (MCT) [51, 52]. Thus, lactate may act as a downstream maker for aerobic glycolysis and hypoxic response in tumors.

1.3.3 Tumor phospholipid metabolism

Choline phospholipid metabolism, also known as the Kennedy pathway, is the major biosynthetic pathway of *de novo* phosphatidylcholine (PtdCho) synthesis in mammalian

cells. PtdCho is the most abundant phospholipid in the cells and forms together with other lipids the characteristic bilayer structure of the cell membrane. Choline-containing metabolites (tCho) include glycerophosphocholine (GPC), phosphocholine (PC), and free choline (Cho), and are involved in cell signaling, lipid metabolism, and the structural integrity of the cell membrane. PC is both a precursor and a breakdown product of PtdCho that can act as a secondary messenger with the ability to stimulate cell proliferation through several growth factors [53], while GPC is a breakdown product of PtdCho that may reflect phospholipid membrane turnover [54]. GPC is also an abundant osmoprotective osmolyte that can protect intracellular macromolecules from being denatured during hypertonic stress in kidney cells [55]. Cho is derived from the diet, but it is also a breakdown product of GPC that is used to synthesize PC. However, the daily requirement of Cho from dietary sources are not well defined [56].

Choline phospholipid metabolism is changed in most cancers, including breast cancer [57, 58]. The tCho levels detected with *in vivo* MR spectroscopy have been suggested as a biomarker for the diagnosis and treatment evaluation of breast cancer [58-62]. However, the molecular mechanisms behind the changes in choline phospholipid metabolism observed within breast cancer are not fully understood. The regulation of choline phospholipid metabolism can be affected by growth factor stimulation, cytokines, hypoxic and inflammatory responses, and oncogenic signaling [63-67]. Choline phospholipid metabolism is a complex pathway controlled by several regulatory enzymes (Figure 1.3). Changes in the expression of genes and enzymes involved in the biosynthetic and catabolic pathways of choline phospholipid metabolism, including choline kinase alpha (CHKA), PtdCho-specific phospholipase D (PLD), and PtdCho-specific phospholipase C (PLC), have been found in different types of cancers [57]. Increased activity of the choline transporters such as organic cation transporter-2 (OCT2) and choline transporter-1 (CHT-1) have also been identified [57]. CHK (E.C. 2.7.1.32) is the enzyme at the first step of the Kennedy pathway which regulates the phosphorylation of Cho to PC. Increased CHK activity has been detected in breast cancer tissue compared to normal healthy tissue [68]. CHK inhibition and down-regulation by RNA interference efficiently decreased cellular PC and tCho levels along with proliferation and tumor growth in human breast cancer cells and animal

models [69-72]. Phosphocholine cytidylyltransferase (CTP, E.C. 2.7.7.15), which uses PC as a substrate to synthesize cytidine diphosphate choline (CDP-choline), has been found to be underexpressed in cancer cells, leading to elevated PC levels [73].

The GPC levels in choline phospholipid metabolism is dependent on the degradation rate of PtdCho by the two enzymes phospholipase A2 (PLA2, E.C. 3.1.1.4) and lyso-phospholipase A1 (LYSO-PLA1, E.C. 3.1.1.5), and the degradation of GPC itself into Cho and glycerol-3-phosphate by glycerophosphocholine phosphodiesterase (GPC-PDE, E.C. 3.1.4.2). The gene expressions of PLA2 and LYSO-PLA1 correlate with GPC levels and can be underexpressed in malignant compared to non-malignant mammary epithelial cells [74]. The gene (s) responsible for GPC-PDE has not yet been identified. Glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) is proposed as a candidate gene for GPC-PDE, as its expression has been shown to affect the enzymatic activity of GPC-PDE and consequently the GPC levels in mouse kidney cells [75]. To date, the role of GDPDs in choline phospholipid metabolism of human breast cancer have not yet been investigated.

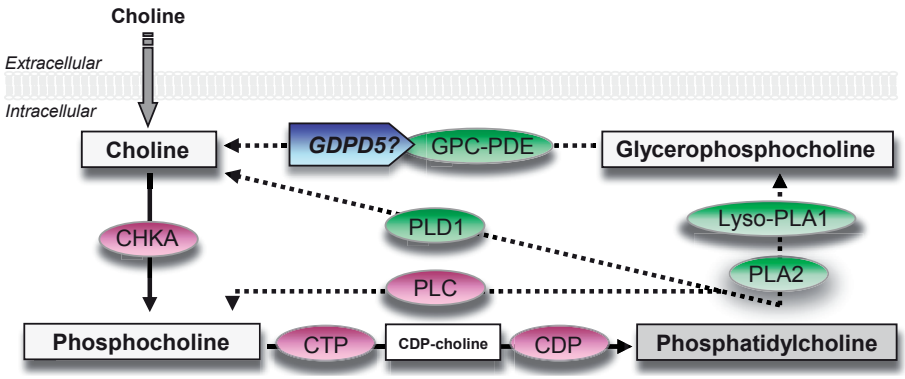

Figure 1.3: The biosynthetic (solid lines) and catabolic (dash lines) pathways of choline phospholipid metabolism. GDPD5 have been suggested as a candidate gene for GPC-PDE. However, its role in choline phospholipid metabolism of human breast cancer is still unknown. CHKA, choline kinase alpha; CTP, phosphocholine cytidylyltransferase; CDP-Cho, cytidine diphosphate choline; CDP, diacylglycerol cholinephosphotransferase; PLA2, phospholipase A2; Lyso-PLA1, lysophospholipase A1; PLD1/PLC, phosphatidylcholine-specific phospholipase D1/C; GPC-PDE, glycerophosphocholine phosphodiesterase; GDPD5, glycerophosphodiester phosphodiesterase domain containing 5.

1.4 Magnetic resonance spectroscopy (MRS)

MRS is an analytical technique that provides identification and quantification of the metabolites. Metabolomics is the systematic study of metabolites present in a living system such as cells, tissues, biofluids, and organs. MRS metabolomics is emerging, and has provided essential information about the biochemical changes in cancers and other diseases [76-79]. Various metabolic biomarkers related to glycolysis and choline phospholipid metabolism have been reported using MRS analyses [19].

The MR spectrum is a plot of the intensity of MR signals versus the magnetic field frequency, usually given in parts per million (ppm). The MR signals stem from the interaction of radiowaves with atomic nuclei. In the presence of an external magnetic field (B_0), atomic nuclei can absorb energy of characteristic frequencies, depending on the physical and chemical environment of the nucleus. In order for a nucleus to give rise to a MR signal, it must have a physical property called spin. The spinning nucleus generates a magnetic moment (μ), which is proportional to its spin quantum number (I).

The most interesting nuclei for biological/medical MRS are hydrogen-1 (^{1}H), carbon-13 (^{13}C), and phosphorous-31 (^{31}P). These nuclei have a spin quantum number I = ½, i.e. they have two possible spin states. The spin states are of low or high energy, as the magnetic moment can be directed with or against the B_0, respectively (Figure 1.4). A small majority of nuclear spins will have their magnetic moment aligned with the B_0 (designated Z-axis). This small excess of nuclei in the low energy state is the basis for the MR signal. By applying a radio frequency (RF) pulse, nuclei in the low energy state will be transferred to the high energy state. The energy difference between the low and

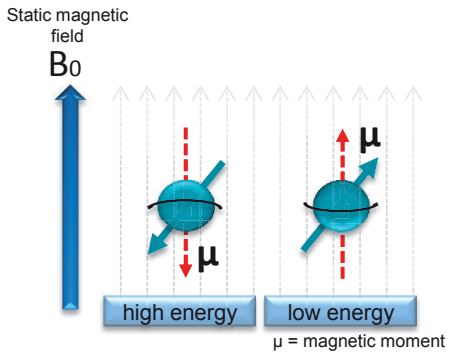

Figure 1.4: In the presence of an external magnetic field (B_0) two spin states exist, the magnetic moment (μ) of low energy state and high energy state.

high energy state is dependent on the strength of the static magnetic field, thus the higher field strength, the higher sensitivity. Following an RF pulse, the exited nuclei interact with their environment, causing them to lose their excess energy and relax back to equilibrium. The emitted energy can be measured, as an MR signal. The nuclei return to equilibrium through processes characterized by two time constants called longitudinal (T_1) and transversal (T_2) relaxation. T_1 describes the time it takes for spinning nuclei to re-align with B_0 and is dependent on the nuclei interactions with the environment. Small molecules have low probability for interaction due to their fast movements which result in long T_1, while lipids and proteins have a higher probability for interaction due to their slow movements and thus exhibit relatively short T_1. The longitudinal magnetization is fully recovered to its equilibrium value after about five times T_1. T_2 describes the time it takes for spinning nuclei to lose phase coherence due to magnetic field inhomogeneities and spin-spin relaxation arising mainly from neighboring nuclei. Large molecules, such as lipids and proteins, have higher interaction probability than smaller molecules and therefore shorter T_2 values. T_2 relaxation times is commonly utilized to reduce the signals from lipids and proteins by applying pulse sequences allowing T_2- weighted MR spectra.

MR spectra signals from solids or semisolids (e.g. tissues) are much broader than those from liquids (e.g. cell extracts). In liquid samples, the molecules are more mobile and the rapid isotropic motions of the nuclei can average the anisotropic nuclear interactions between the nuclei and effectively remove them from the spectrum, which results in spectra with narrow line width (Figure 1.5). In solid samples, the lack of molecular mobility leads to anisotropic nuclear interactions, such as magnetic dipolar interactions, electric quadrupolar, and electron shielding interactions between the nuclei. These interactions result in large anisotropic broadenings and spectra with broad and overlapping signals [80].

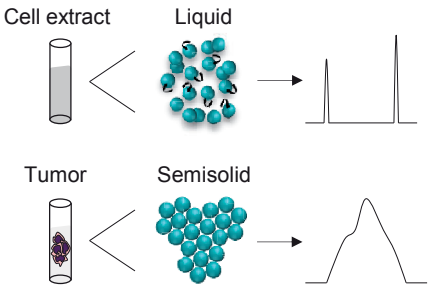

Figure 1.5: Liquid and semisolid MRS

1.4.1 High resolution magic angle spinning (HR MAS)

For nuclei with spin quantum number I = ½ (e.g. ^{1}H, ^{13}C, ^{31}P), line-narrowing in the MR spectra of solid samples can be achieved by imposing a motion on the nuclei which removes anisotropic sources of broadening. The principle of magic angle spinning (MAS) was first described by Andrew and Lowe in 1958 [81, 82]. Rapidly spinning of the solid samples at the magic angle, referring to the angle of 54.7 degrees, will mimic the motion of nuclei in a liquid state; resulting in sharp and highly resolved spectra, comparable with those from liquids.

When a sample is spun about an angle to B_0, the dipole interaction between the nuclei is dependent on the angle and the spin-rate (ω_r) (Figure 1.6). When the angle is 54.7 degrees, line broadening in solids can be reduced as some of the dipolar interactions are eliminated. Spinning the samples splits the broad signals into narrow lines, but it will also introduce low-intensity lines in the MR spectra that are spin-dependent, the so-called spinning sidebands. The intensity of the sidebands decreases with increasing spin rate. However, high-speed spinning can lead to destruction of tissue structures. The spin rates are chosen to eliminate the sidebands from the spectral region of interest. A wide range of spin rates, spanning from 1 Hz to 12 kHz, has been used for high-resolution magic angle spinning (HR MAS) studies. For samples analyzed with a 600 MHz spectrometer, a spin rate of 5 kHz provides good spectral quality while preserving the tissue structures [83].

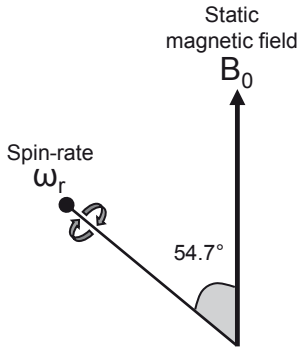

Figure 1.6: Schematic illustration of magic angle spinning MRS

Ex vivo ^{1}H HR MAS MRS has been commercially available since the late 1990's. The technique is non-destructive and requires no difficult sample preparation before analysis. The sample remains intact for further analyses such as histopathology, proteomics, and transcriptomics, thus allowing for a comprehensive and detailed study of the biochemical composition of the tissue. This has contributed to an improved

understanding of biochemical changes that occur in cancer and during cancer treatment. It has been applied to several types of human cancers including brain [84, 85], breast [83, 86], and prostate [87, 88]. In addition, several studies have proven ^{1}H HR MAS MRS as a promising tool for diagnosis and treatment monitoring of breast cancer [89, 90].

1.4.2 Analyses of breast cancer spectra

^{1}H HR MAS MRS metabolite profiles of breast cancer tissue have been shown to correlate to parameters of clinical importance, such as tumor grade, axillary lymph node status, and hormone receptor status [39, 91, 92]. More than 30 metabolites have been detected and assigned in breast cancer tissue [83]. Part of a ^{1}H HR MAS MR spectrum including some of the most studied metabolites in breast cancer tissue is shown in Figure 1.7. Different approaches can be used to extract information from the MR spectra, of which multivariate data analyses and metabolite quantification are most common.

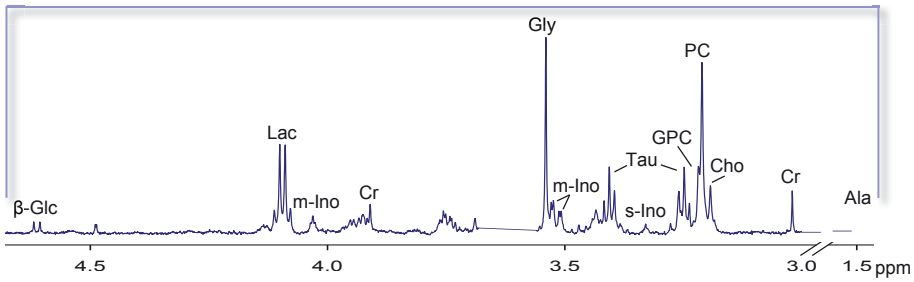

Figure 1.7: An example of the metabolite profile of breast cancer tissue obtained by ^{1}H HR MAS MRS with the spectral region that was used in paper II. The signals from water, large lipid residuals, and ethanol contamination were removed prior to multivariate data analyses. β-Glc, beta glucose; Lac, lactate; m-Ino, myo-Inositol; Cr, creatine; Gly, glycine; Tau, taurine; s-Ino, scyllo-Inositol; GPC, glycerophosphocholine; PC, phosphocholine; Cho, free choline; Ala, alanine.

Multivariate data analysis

One of the many challenges for the analysis of ^{1}H HR MAS MR spectra is the large number of variables (points in the MR spectra) compared to the low number of samples (individuals). Multivariate statistical methods are specialized to handle such data sets, and can be used in a reliable manner to identify biomarkers and/or discover metabolic features related to the discrimination between distinct classes.

Preprocessing

Spectral preprocessing is a crucial step for building a precise and accurate multivariate model. There are many available methods for preprocessing the MR data. The chosen algorithms and parameter settings should be optimized to increase the quality of the spectra for multivariate modeling. Preprocessing methods including baseline correction, normalization, and peak alignment is usually recommended [93-95]. Mean normalization (also called area normalization) is a common method used to achieve an equal total area for each spectrum, without altering the relative intensities of the metabolite signals within the spectrum. This is used to compensate for individual differences in the samples, such as sample weight and total concentration of the metabolites. The peaks of MR spectra are usually not well aligned due to small variations in pH, temperature, and intermolecular interactions in the samples. Different peak alignment algorithms are available for correcting peak misalignment between the MR spectra, e.g. SpecAlign, *i*coshift, and COW [96-98]. In general, the algorithms are based on the insertion and deletion of spectral data points or intervals to shift regions in the spectrum to align with the corresponding region in a reference spectrum.

Variable selection for multivariate modeling can be performed by a genetic algorithms (GA), which is a supervised variable selection method based on the principles of genetics and natural selection [99, 100]. GAs generate a population comprising randomly subsets of selected variables (so-called ‘chromosome’) and uses them to produce the next generation via reproduction and mutation. The process is repeated a number of generation until an optimal population consistent of the most useful subsets of variables has been developed.

Multivariate methods

Multivariate statistic methods such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) can be used for finding linear relationships between the spectral data and clinical features, such as treatment response [101]. PCA explains the underlying variance structure of a data set through linear combinations of the variables. These linear combinations are called principal components (PCs). The first PC (PC1) covers the maximum variation of the data structure, PC2 covers the second largest variation in the data, but is completely uncorrelated to PC1 (orthogonal direction). Each following component accounts for as much of the remaining variability as possible. The goal of PCA is to filter out the noise and redundant data to compute the most important variance structure of the data, usually described by the few first PCs. By plotting the PCs against each other in a score plot, the hidden interrelationships between different variables can be revealed. The loadings show how much each variable contributes to each PC. The corresponding score and loading plots are complementary and can be use to interpret the model. PCA is an unsupervised method, thus it explains the experimental data (e.g. spectral data) without taking into consideration any other relevant information such as clinical data. However, PCA is useful for the initial description of sample patterns and to detect outliers before carrying out more sophisticated multivariate data analyses.

PLS-DA is a supervised data compression method used to detect the relationships between two matrices; experimental data (**X**) and the response variables (**Y**). PLS-DA aims to find underlying structures, called latent variables (LVs) that maximize the covariance between **X** (e.g. spectral data) and **Y** (e.g. treatment response). The **Y**-matrix consists of so-called "dummy variables", classifying each sample in this example as either responders (-1) or non-responders (+1). In a similar manner as for PCA, the corresponding score and loading plots are used to identify the differences in the metabolite profile between classes, and subsequently to discover potential biomarkers for the clinical feature being investigated, see Figure 1.8. The variable importance in the projection (VIP) scores can be used to assist the identification of metabolites of importance for the classification [102].

Multilevel PLS-DA is an extension of the ordinary PLS-DA and can be used to investigate the paired structure within the different subjects [103, 104]. This analysis can only be applied for data with multilevel structure, i.e. when interventions are evaluated on the same subject. In multilevel PLS-DA, the *between* subject variation resulting from differences in age, disease state, genetics, and other factors, is separated from the *within* subject variation, thus enabling the detection of metabolic changes caused by the intervention. The between subject variation is described by the average of the two observations from one subject, whereas the within subject variation is described by the net difference between them [103]. After the split-up of variation, ordinary PLS-DA classifications of the within or between subject variations can be performed.

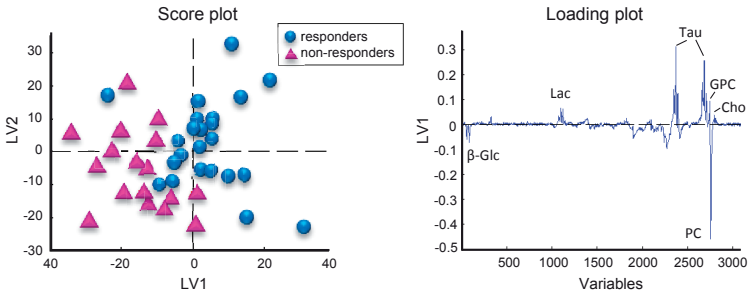

Figure 1.8: A constructed example of the PLS-DA score and loading plots of responders and non-responders to a given treatment. The score plot shows the sample inter-relationships, thus similar samples are located close to each other. The loading plot shows the variable inter-relationship and is used to interpret the reasons behind the object distribution. The scores and variables close to zero have no influence on the classification. In this example, the different classes are well discriminated by latent variable 1 (LV1). Responders have positive scores for LV1 and appear to have more of the metabolites lactate, taurine, GPC and Cho, while non-responders have negative scores for LV1 higher levels of PC and β-glucose. β-Glc, beta glucose; Lac, lactate; Tau, taurine; GPC, glycerophosphocholine; PC, phosphocholine; Cho, free choline.

Validation

Validation of multivariate models is necessary to measure the robustness of the models and to find the optimal dimensionality in order to avoid either overfitting or underfitting. Validation methods such as cross-validation, test set data, and permutation testing are used to assess the significance of the models. During validation the dataset is split in two parts; (1) a training set for model calibration and (2) a test set for model validation. In "leave one out" or full cross-validation only one sample is used for testing at a time, while the remaining samples (*n*-1 when there are *n* observations) are used for training. The procedure must be repeated until all samples have been validated once. The classification result for each sample is summed and the average represents the validated classification result of the model. Full cross-validation may give too optimistic results due to data overfitting, but is a convenient choice for data sets with low sample numbers (n~20). For larger data sets, validation by test set data using a defined percentage of the samples for testing and the remaining for training is more appropriate. Limiting the number of LVs is necessary in order to achieve a reliable model. The minimum number of LVs with the minimum classification error determined by cross-validation can be used to optimize the number of LVs.

A permutation test can be used to evaluate the statistical significance of the classification results [101]. Permutation testing is performed by randomly assigning the class labels to the samples. The permutation procedure is then repeated a number of times and for each time the permuted classification result is calculated. The classification result of the original model can then be compared to the distribution of classification results from the permutation procedure.

Metabolite quantification

MRS is a powerful tool for quantification of metabolites present in a sample since the area of a given signal in the MR spectrum is proportional to the number of nuclei responsible for that signal. The area of a reference signal with known concentration can thus be used to perform absolute quantification. Accurate determination of metabolite concentrations can provide important biochemical information for a better understanding of cancer behavior and progression.

Quantification of metabolites in intact breast cancer tissue is highly challenging due to the inherent complexity of the spectra and overlapping spectral signals from many metabolites. Moreover, the signals are also overlapping with the macromolecules like proteins and lipids. Peak integration is a simple and widely used method for peak area measurement in MR spectra. However, due to overlapping signals, more advanced model fitting methods should be applied, such as curve fitting. PeakFit (PeakFit v 4.12, SeaSolve Software Inc.) is a software that uses nonlinear curve fitting method to calculate the area of overlapping metabolite signals in a spectrum (Figure 1.9) [105]. Prior knowledge of the overlapping metabolite signals is essential to increase the quality of the fitting.

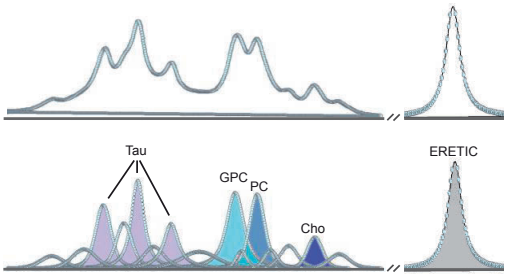

Figure 1.9: Metabolite quantification of a ^{1}H HR MAS spectrum using a peak fitting algorithm (e.g. PeakFit) and an ERETIC reference signal. PeakFit enables the calculation of area from overlapping signals and by using the ERETIC as a reference absolute concentration of individual metabolites can be quantified. Tau, taurine; GPC, glycerophosphocholine; PC, phosphocholine; Cho, free choline; ERETIC; electronic reference to access in vivo concentration.

The use of a reference compound for absolute quantification of metabolites in tissue specimens is also challenging. An ideal reference for tissue metabolite quantification should be highly reproducible, not react chemically with the sample, and resonate away from the metabolite signals of interest. Trimethylsilyl propionic acid (TSP) is commonly used as an internal reference in ^{1}H HR MAS spectroscopy of tissue [106]. However, the hydrophobic chain of TSP tends to interact with proteins and other membrane components present causing an overestimation of metabolite concentrations [107]. TSP is therefore more suitable as a reference for cell and tissue extracts than tissue samples.

Several studies have used the internal tissue water signal as a reference measured in an additional experiment without water suppression [84, 87]. However, the unsuppressed water signal is typically 1,000-10,000 times larger than most metabolites in the tissue. In addition, the relaxation time and the density of water are not constant in all tissues and tend to change in pathologies [108]. Therefore, extra consideration should be taken when evaluating the quantitative results using internal water as reference.

The electronic reference to access in vivo concentration (ERETIC) published in 1999 by Akoka *et al.* is a promising alternative to the use of an internal reference [109]. The ERETIC signal generates a "synthetic" peak of which the MR characteristics, e.g. frequency, linewidth, and magnitude, are modifiable. In addition, there are no concerns about metabolic activity. Furthermore, the ERETIC signal can easily be moved away from the metabolite region of interest [109, 110]. Calibration of the ERETIC signal to a standard of known concentration (e.g. sucrose or creatine) then permits the use of this signal to quantify metabolites of interest in the sample [110-112].

1.5 Gene and protein analyses

The combination of gene, protein and metabolite data is an advantage for the investigation of molecular pathway regulation. Quantitative real-time polymerase chain reaction (qRT-PCR) is a powerful and sensitive gene analysis technique for detection and quantification of a specific target sequence. Real-time detection of PCR products is made possible by using two oligonucleotide primers, forward and reverse, which are designed to specifically bind to the target sequence and a fluorescent molecule that reflects the amount of amplified PCR product. qRT-PCR data can be evaluated without gel electrophoresis resulting in reduced experimental time and increased throughput. Relative quantification of the gene expression can be determined by relating the PCR signal to a reference group, such as untreated controls or normal samples. Immunoblotting (Western blotting) allows for the identification of proteins according to their molecular weight by gel electrophoresis. The proteins are detected by using protein specific antibodies (Figure 1.10).

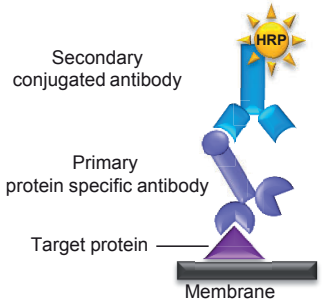

Figure 1.10: The principle of Western blotting analysis. Proteins are separated and transferred from the gel to a membrane. The membrane is exposed to the primary protein specific antibody in the present of a protein-rich solution to reduce non-specific binding. After several washing steps, the membrane is then exposed to the secondary antibody conjugated to a detectable molecule, e.g. horseradish peroxidise (HRP), which produces a colored band when incubated with the appropriate substrate.

2 Aims

The main objective of this thesis was to explore the metabolite profiles of locally advanced breast cancers and the effects of NAC. More specific, the goals were to:

- Evaluate whether MR metabolic profiling can assist the prediction of clinical treatment response in LABC patients receiving NAC

- Investigate the prognostic value of the MR metabolite profile in LABC patients

- Investigate the role of GDPDs in choline phospholipid metabolism of breast cancer cell lines and patient samples

3 Materials and methods

The present thesis includes three papers presenting data from MR metabolomics, and gene and protein analyses of human breast cancer tissue and cell lines. A summary of the materials and methods used in the thesis is given in Table 3.1.

Table 3.1 Materials and methods used in the papers I-III.

			Paper I	Paper II	Paper III
Materials		Human samples	Tissue samples n=33p/66s	Tissue samples n=89p/178s	Tissue samples n=19s
		Cell lines	-	-	MCF-12A (n=7) MCF-7 (n=10) MDA-MB-231 (n=8)
Methods		Tumor cell content	Histology sections	Imprint cytology	Imprint cytology
		Metabolomics	^{1}H HR MAS MRS	^{1}H HR MAS MRS	^{1}H HR MAS MRS/ ^{1}H HR MRS
		Gene expression	-	-	qRT-PCR
		Protein expression	-	-	Western blotting
Data analyses		Metabolite quantification	PeakFit reference: ERETIC	Relative intensity	PeakFit/Integration reference: ERETIC/TSP or TMS
		Multivariate modeling	PLS-DA	Multilevel PLS-DA	-
		Gene and protein expression	-	-	Relative fold change

p, pair (pre- and post-treatment); s, samples; ^{1}H HR MAS MRS, proton high-resolution magic angle spinning magnetic resonance spectroscopy; qRT-PCR, quantitative real-time polymerase chain reaction; ERETIC, electronic reference to access in vivo concentration; TSP, trimethylsilyl propionic acid; TMS, tetramethylsilane; PLS-DA, partial least squares discriminant analysis.

3.1 Human breast cancer patients and cell lines

The patients

LABC patients (n=122) included in the three presented papers were enrolled in two large clinical studies evaluating predictive factors for response to NAC. The studies were approved by the Regional Ethical Committee (Norwegian Heath Region III) and each patient gave written informed consent. The detailed description of the inclusion and treatment protocols is fully described elsewhere [113, 114]. In brief, female patients diagnosed with invasive breast cancer stage IIIA-C and a subset of stage IIB defined as T3-T4 (any N) or N2-N3 (any T) without distant metastasis (M0) were enrolled in 1991-2003. Patients with minor metastatic deposit (e.g. in the bone and liver) in addition to their locally advanced disease at the time of diagnosis were categorized as stage IV disease. These patients were also included in the studies providing they were candidates for surgical treatment of their primary local tumor after NAC. Patients with IBC were excluded in both studies due to their distinct clinical presentation and poor prognosis. Breast cancer tissue samples were obtained from an open biopsy procedure before NAC and during final surgery. The breast cancer tissue samples were snap-frozen immediately (~ 1 min) after removal and stored in -80°C.

Patient treatment protocols

All patients included in the studies received NAC. In paper I, the patients were treated with weekly doxorubicin (14mg/m^2) for 16 weeks before surgery. In paper II, first line treatment with four doses of epirubicin (90 mg/m^2) or paclitaxel (200 mg/m^2) was administrated to the patients every third week. In case of insufficient response or the tumor was still inoperable after four doses, cross-over to second line treatment or extension of four doses of the same first line treatment was given. In paper III, only pre-treatment breast cancer tissue samples were used. All patients underwent a mastectomy and post-operative radiation therapy. Patients with hormone receptor positive tumors (ER and/or PgR $\geq$ 10 fmol/mg protein [115] or $\geq$ 10% positive cells detected with immune histochemistry) were treated with tamoxifen for five years after surgery.

Clinical treatment response and breast cancer survival

Clinical tumor response was evaluated by comparing caliper measurements pre- and post-treatment and assigned according to the WHO criteria (Table 1.2). Patients included in paper I and II had either a partial response or stable disease in response to NAC. Survival time was defined as the time interval from diagnosis until the end of the observation period (five years). Patients deceased within five years after diagnosis were classified as non-survivors whereas patients surviving five years or more were classified as survivors. Patients that died of other causes than breast cancer were censored.

The cell lines

All cell lines included in paper III were obtained from American Type Culture Collection (ATCC, www.atcc.org). Non-malignant MCF-12A and two malignant human mammary epithelial cell lines, MCF-7 and MDA-MB-231, were used in the cell experiments. MCF-7 is an estrogen-sensitive and weakly malignant, while MDA-MB-231 is an estrogen-independent and highly malignant breast cancer cell line. The cells were grown in their respective media supplemented with fetal bovine serum (FBS), penicillin, and streptomycin as previously described [116]. All cells were cultured at 37°C in 5% CO_2 and were used while sub confluent (approximately ~70%).

Tumor cell content

In paper I, breast cancer tissue samples were first analyzed by ^{1}H HR MAS MRS before formalin-fixed, embedded in paraffin, and stained with hematoxylin-eosin-saffron (HES). In paper II and III, imprint cytology smears were prepared from each tissue sample and stained with May-Grünwald-Giemsa prior to ^{1}H HR MAS MRS. The tumor cell content was determined microscopically by a pathologist. For cells in culture (paper III), trypan blue staining was used as vital stain for cell counting.

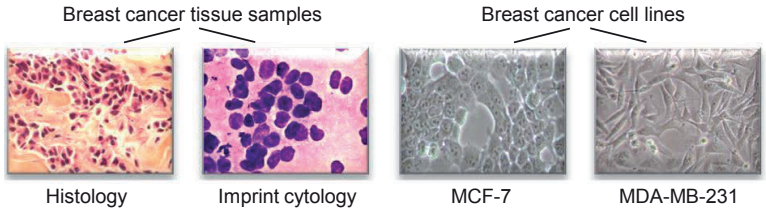

Figure 3.1: Microscopic images of breast cancer tissue samples and cultured cells.

3.3 ^{1}H HR MAS and ^{1}H HR MRS experiments

Sample preparation

Patient tissue samples were blinded and randomized prior to ^{1}H HR MAS MRS analysis (http://www.random.org/lists/). The samples were randomized based on chemotherapy agents (paper II), clinical treatment response, and pre- and post-treatment samples. Pair of samples (pre- and post-treatment) from each patient were analyzed in series to minimize experimental artifacts. Tumor tissue (15.5±3.0 mg) and phosphate buffered saline (PBS, 3 µl) in D_2O containing 98.2 mM TSP for chemical shift referencing were added to a 30 µl leak-proof disposable insert (Bruker Biospin Corp, USA) and transferred to a MAS rotor. ^{1}H HR MAS spectra were obtained using a Bruker AVANCE DRX600 spectrometer (Bruker Biospin GmbH, Germany). Samples were spun at 5 kHz at 4°C to minimize tissue degradation. Spin-echo spectra (cpmgpr; Bruker) were recorded for all the samples as previously described [39]. In addition, a single pulse experiment including the ERETIC signal (ereticpr.drx; Bruker) as a quantification reference [111] was performed for samples included in paper I and III.

For cell cultures, the lipid and water-soluble metabolites were extracted using a dual-phase extraction method based on methanol/chloroform/water as previously described [74]. The water-soluble and lipid fractions were dissolved in deuterated solvents containing 0.24×10^{-6} mol TSP and 2.17×10^{-7} mol tetramethylsilane (TMS) respectively, as quantification references and chemical shift standards. Fully relaxed ^{1}H HR MRS spectra were obtained using a Bruker AVANCE 500 spectrometer (Bruker Biospin Corp).

MRS protocols

The different MRS protocols for the recorded spectra are summarized in Table 3.2.

Table 3.2 MRS protocols

Method	**Tissue samples** ^{1}H HR MAS MRS		**Cultured cells** ^{1}H HR MRS
Spectrometer	Bruker advanced 600 (14.1 T)		Bruker advanced 500 (11.7 T)
Pulse program	Spin-echo	Single pulse with ERETIC reference	Single pulse
	cpmgpr; Bruker	ereticpr.drx; Bruker	zg; Bruker
Parameters	**Paper I + II + III**	**Paper I + III**	**Paper III**
Flip angle	90°	60°	45°
NS	128	128	128
Time domain size	32K points	64K points	32K points
AQ	1.64 s	3.28 s	0.819 s
Sweep width	10 kHz/16.7 ppm	10 kHz/ 16.7 ppm	6 kHz/ 12.0 ppm
TE	285 ms	-	-
TR	3.93 s	18.28 s	12.70 s

NS, number of scans; AQ, acquisition time; TE, total echo time; TR, repetition time.

The different acquisition times (AQ) used in the spin-echo and ERETIC pulse programs are appropriate for breast tissues. The single pulse spectra with the ERETIC reference of tissue samples and single pulse spectra of cultured cells were acquired with short flip angles (60° and 45°, respectively) and long repetition times to avoid saturation of the signals. The pulse sequence was repeated (NS=128) to achieve a good signal-to-noise ratio (S/N). The spin-echo sequence was used to suppress signals with short T_2 values (e.g. lipids and macro molecules). A total echo time (TE) of 285 ms was obtained by using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with 1 ms echo delay and 136 loops (Figure 3.2). Zero-filling was applied to increase the digital resolution (paper I-III, tissue samples). Spectra were Fourier transformed into 128 K after 0.3 Hz line broadening and chemical shifts were calibrated to the TSP signal at 0 ppm (paper I-III, tissue samples).

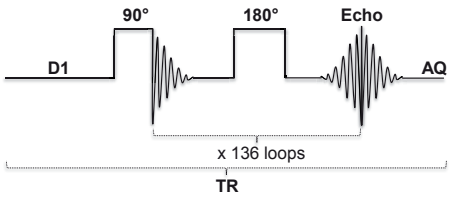

Figure 3.2: Schematic illustration spin-echo sequence used in paper I-III.

3.4 Gene and protein experiments

In paper III, total RNA isolation from human breast cancer tissue samples and cultured cells was performed using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. The concentration and purity of RNA yield were determined with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). In addition, the integrity of RNA (RIN) of the tissue samples was measured using Bionalyzer 2100 (Agilent). Total RNA (1 µg) was used for preparation of cDNA by SuperScript III First-Strand Synthesis SuperMix (Invitrogen). The mRNA levels of GDPD1-5, CHKA, and PLD1 were measured by qRT-PCR using the iCycler real-time PCR detection system (Bio-Rad) and iQ SYBR Green Supermix (Quanta BioSciences). Gene specific primers (Invitrogen) were designed using the Primer-BLAST tool (http://www.ncbi.nlm.nih.gov). The relative fold change (R) in gene expression was calculated based on the threshold cycle (ct) using the ΔΔct method as $R = 2^{-\Delta(\Delta ct)}$ [117], where Δct = ct(target gene) − ct(housekeeping gene), and $\Delta(\Delta ct) = \Delta ct$(experiment sample) − Δct(control sample).

Protein isolation was carried out using lysis buffer containing a protease inhibitor cocktail (P8340, Sigma-Aldrich). GDPD5 Western blotting for the three tested cell lines was performed as previously described [116]. The proteins were denatured and separated by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis). Thirty micrograms of total protein, as determined by a modified Lowry assay (Bio-Rad), was loaded in each lane, and two lanes were loaded with molecular weight standard (BenchMark, Life Technologies). The proteins were transferred to a membrane and incubated for 1 hour with a dilution (1:500) of primary polyclonal anti-GDPD5 antibody (Cat. No. AP10992c, Abgent Inc.) followed by incubation with horseradish peroxidase (HRP) conjugated secondary antibody (Amersham Life Science). Visualization was performed using the Supersignal West Pico chemiluminescent substrate kit (Pierce Biotechnology). The membrane was recorded on Blue Bio film (Denville Scientific). Quantitative densitometry of the GDPD5 proteins was performed using the Gel-analysis-tool in ImageJ (Wayne Rasband, NIH, Bethesda, MD). We did not have sufficient tumor material to run immunoblots on the patient samples.

3.5 Data analyses

Tissue samples with low tumor cell count and poor spectral quality were excluded from further analyses.

3.5.1 Multivariate data analyses

The different preprocessing and validation methods of spin-echo spectra used for multivariate data analyses are presented in Table 3.3. Prior to spectral preprocessing, signals from water, large lipid residuals, and ethanol contamination were removed from the spectra. Data analyses were performed in Matlab R2009a (The Mathworks, Inc.) using PLS_Toolbox 6.2.1 (Eigenvector Research).

Table 3.3 Multivariate data analyses of spin-echo spectra

	Paper I	**Paper II**
Preprocessing methods	PLS-DA/ GA PLS-DA	Multi level PLS-DA/ PLS-DA
Average	Reduced by a factor of 2	Reduced by a factor of 2
Baseline correction	Baseline offset	Asymmetric least squares and baseline offset
Normalization	Equal total area	Equal total area
Peak alignment	SpecAlign	*i*coshift
Mean centering	yes	yes
Variable selection	Genetic algorithm	-
Validation methods		
Validation	Full cross-validation	10% random test set
Permutation	-	yes

PLS-DA, partial least squares discriminant analysis; GA, genetic algorithm.

Spectral data from each sample were reduced by a factor of 2 using the mean value of two consecutive data points. Baseline offset was corrected by shifting the minimum intensity value of each spectrum to zero. In paper II, asymmetric least squares baseline correction was used in addition to baseline offset [118]. The spectra were peak aligned using SpecAlign algorithm with the mean spectrum serving as reference (paper I) [96] and *i*coshift (paper II) [97]. Mean centering was performed by subtracting the average spectrum of all the spectra from each spectrum. GA variable selection prior to PLS-DA was performed in paper I.

In paper I, full cross-validation was used due to the low number of samples ($n \leq 26$). In paper II, validation was performed with randomly chosen test set using 90% of the patients for training and the remaining 10% for testing. This procedure was repeated 20 times and the average validated classification results were calculated. To avoid biased results, the same number of LVs was used for all repetitions and was chosen by cross-validation of the whole data set. The VIP scores were used to assist the interpretation of the PLS-DA loadings. Permutation testing was performed by randomly assigning the class labels to the samples and the average results were calculated as described for the original data (90% training set, 10% test set, 20 repetitions). The permutation procedure was repeated 1000 times in which the class labels were reshuffled each time. The classification result of the original data was compared to the distribution of classification results from the permutation. P-values ≤ 0.05 were considered significant.

3.5.2 Metabolite quantification

The single pulse spectra with the ERETIC reference of tissue samples and single pulse spectra of cultured cells were used for metabolite quantification. Tissue metabolite quantification using curve fitting (PeakFit v 4.12, SeaSolve Software Inc.) and the ERETIC signal as reference was performed in paper I and III. The metabolite concentration was calculated using Equation I [111]. In paper III, metabolite quantification of cell extracts was determined by peak integration (MestReC 4.9.9.6 software, Mestrelab Research) using Equation II [74]. The signal integrals of TSP and TMS were used as references for the water-soluble and lipid extracts, respectively. In paper II, relative metabolite intensities were calculated by integrating the peak areas of spectra normalized to equal total areas after removal of signals from water, lipid residuals, and ethanol contamination (Matlab R2009a, The Mathworks, Inc.).

Equation I, tissue samples

$$[MET]\,\text{mol/g} = \frac{A_{MET}}{A_{ERETIC}} \times \frac{1}{k_{MET}} \times \frac{n_{ERETIC}}{m_{sample}}$$

A_{MET}: metabolite area
A_{ERETIC}: ERETIC area
k_{MET}: number of protons of the metabolite signal
n_{ERETIC}: number of moles in the ERETIC signal
m_{sample}: sample mass (gram)

Equation II, cell extracts

$$[MET]\,\text{mM} = \frac{I_{MET}}{I_{TSP/TMS}} \times \frac{1}{k_{MET}} \times \frac{n_{TSP/TMS}}{m_{cell}} \times \frac{1}{m_{volume}}$$

I_{MET}: metabolite integral
$I_{TSP/TMS}$: TSP/TMS integral
k_{MET}: number of protons of the metabolite signal
$n_{TSP/TMS}$: number of moles in the TSP/TMS signal
m_{cell}: cell count
m_{volume}: cell volume

Statistical analyses were performed using SPSS (SPSS 16.0 Inc.). The Kolmogorov-Smirnov test was used to evaluate normality of the data. For normal distributed data, the statistical differences between multiple groups and paired samples were determined using two-tailed unpaired and paired T-test, respectively. For non-normal distributed data, the grouped Mann-Whitney and paired Wilcoxon Signed Ranks tests were used. P-values of ≤ 0.05 were considered to present significant differences between groups.

In paper I, receiver operating characteristic (ROC) curve analysis was performed using metabolite concentrations obtained from pre-treatment tumor tissue samples to define cut-off levels for prediction of treatment response and breast cancer survival.

3.5.3 Gene and protein expressions

The Grubbs (GraphPad Software) test was performed to detect significant statistical outliers for each gene set (GDPD1-5, CHKA, and PLD1) of the patient tumor samples (significant level alpha ≤ 0.05). Maximally one outlier was discarded per set. The normality of the data was evaluated by the Kolmogorov-Smirnov test (SPSS 16.0 Inc.). The unpaired two-tailed T-test (SPSS 16.0 Inc.) was performed to detect significant differences between the tested groups. Pearson correlation (SPSS 16.0 Inc.) was calculated to investigate the linear correlations between GDPD1-5 gene expressions and choline phospholipid metabolites in human breast tumors and cell lines, but also to examine the correlations between GDPD5 *versus* CHKA and GDPD5 *versus* PLD1 gene expressions. In human breast cancer cells, GDPD5 protein levels were also related to choline phospholipid metabolite and CHKA protein levels. P-values of ≤ 0.05 were considered to be significant.

4 Summary of papers

Paper I

Predicting long-term survival and treatment response in breast cancer patients receiving neoadjuvant chemotherapy by MR metabolic profiling

This paper aimed to evaluate whether MR metabolic profiling can be used for prediction of long-term survival (≥ 5 years) and monitoring of treatment response in LABC patients receiving doxorubicin monotherapy.

^{1}H HR MAS MR spectra of pre- and post-treatment tissue samples from LABC patients (n=33) were acquired. Tissue metabolite concentrations of PC, GPC, Cho, glycine, and taurine were quantified using PeakFit and the ERETIC signal as reference. ROC curves analysis was performed to define their potential to predict patient survival and treatment response. The MR metabolite profiles were related to long-term survival and treatment response by PLS-DA and PLS-DA with GA variable selection.

A significant decrease in GPC post-treatment was associated with long-term survival ($p=0.046$) and partial response ($p=0.014$) to NAC. Long-term survival was best predicted by GPC using ROC analysis (sensitivity 66.7%/specificity 62.5%), while taurine had the best predictive value of clinical treatment response (sensitivity 72.7%/ specificity 63.2%). GA PLS-DA multivariate classification models successfully discriminated between patients with long-term survival (≥ 5 years, survivors) and patients who died of breast cancer recurrence (< 5 years, non-survivors), resulting in 82.7% and 90.2% CV classification accuracy, pre- and post-treatment, respectively. The pre-treatment MR metabolite profiles of survivors were characterized by higher levels

of tCho and lower levels of lactate compared to non-survivors. Classification of clinical treatment response using GA PLS-DA was not successful for this patient cohort.

Our results demonstrate that ^{1}H HR MAS MR metabolic characteristics of breast tumors could potentially assist the classification and prediction of long-term survival in LABC patients, in addition to being used for evaluation of treatment response to NAC.

Paper II

Prognostic value of metabolic response in breast cancer patients receiving neoadjuvant chemotherapy

The aim of this paper was to examine the tumor metabolic changes in patients with LABC caused by NAC, and relate these changes to long-term survival (≥ 5 years) and clinical treatment response.

LABC patients (n=89) participating in a randomized multicenter study were allocated to receive either NAC as epirubicin or paclitaxel monotherapy. Tumor tissue samples obtained pre- and post-treatment were analyzed by ^{1}H HR MAS MRS. MR metabolite profiles were examined by PLS-DA and paired PLS-DA (or multilevel PLS-DA). The metabolites most important for the classification were identified by VIP scores and quantified by spectral integration of the metabolite peaks.

All patients had a significant metabolic response to NAC, and the pre- and post-treatment spectra could be discriminated with 68.9% and 87.9% CV classification accuracy by PLS-DA and multilevel PLS-DA, respectively ($p<0.001$). The metabolic responses appear to be similar for the two chemotherapeutic agents. Different metabolic responses to NAC were observed in patients with long-term survival (≥ 5 years, survivors) and patients who died of breast cancer recurrence (< 5 years, non-survivors). Survivors experienced a decrease in the levels of GPC ($p<0.001$), PC ($p<0.001$), Cho ($p=0.013$), glycine ($p=0.047$), and increase in glucose ($p=0.002$) levels in response to

NAC, while non-survivors had an increase lactate (p=0.004) levels in response to NAC. The metabolic responses to NAC were similar in the different clinical treatment responses.

To conclude, the tumor metabolic response to NAC was related to breast cancer survival, but not to clinical treatment response. Monitoring metabolic responses to NAC by ^{1}H HR MAS MRS may provide information related to individual prognosis.

Paper III

Glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) expression correlates with malignant choline phospholipid metabolite profiles in human breast cancer

Choline phospholipid metabolism is altered in cancer. The gene(s) encoding for the GPC-PDE(s) responsible for GPC degradation in breast cancers have not yet been identified. In this paper, we aimed to investigate the role of GDPDs in choline phospholipid metabolism in breast cancer.

Two human breast cancer cell lines (n=8 and 10) and primary human breast tumor samples (n=19) were studied with combined MRS and qRT-PCR to investigate the potential role of GDPD1-5 as GPC-PDE encoding genes and to determine their relationship with CHKA and PLD1 in choline phospholipid metabolism.

Out of five GDPDs tested, GDPD5 was found to be significantly overexpressed in highly malignant ER^- compared to weakly malignant ER^+ human breast cancer cells (p=0.027) and breast tumors from patients (p=0.015). GDPD5 showed significantly positive correlations with PC (p<0.001), total choline (tCho) (p=0.007) and PC/GPC (p<0.001) levels in human breast tumors. GDPD5 showed a trend towards negative correlation with GPC levels (p=0.130). Human breast cancers with malignant choline metabolite profiles consisting of low GPC and high PC levels highly co-expressed

GDPD5, CHKA, and PLD1, while cancers containing high GPC and relatively low PC levels displayed low co-expression of GDPD5, CHKA, and PLD1. GDPD5, CHKA and PLD1 were significantly overexpressed in highly malignant ER^- tumors in our patient cohort.

Our study identified GDPD5 as a GPC-PDE that likely participates in regulating choline phospholipid metabolism in breast cancer, which possibly occurs in association with CHKA and PLD1.

5 Discussion

The metabolic characterization of breast tumors by ^{1}H HR MAS MRS may assist the prediction of long-term survival in LABC patients (paper I and II). The tumor metabolic responses to NAC were related to breast cancer survival, but not to clinically measured treatment response (paper II). High levels of lactate and glycine post-treatment were associated with poor outcome, while decreased choline phospholipid metabolite levels were observed in response to NAC in patients with long-term survival (paper I and II). Furthermore, GDPD5 was identified as a possible regulatory gene in choline phospholipid metabolism in breast cancer, and high co-expression of GDPD5, CHKA, and PLD1 were associated with increased malignancy (paper III).

5.1 Patients and tumor samples

The tumor samples obtained from open biopsies before NAC and during final surgery were collected from LABC patients from 1991 to 2003. All samples were immediately (~ 1 min) snap frozen after removal and stored in -80°C until used for ^{1}H HR MAS MRS analysis in 2008 (paper I) and 2009 (paper II and III). No significant correlations have so far been found between storage time and tissue metabolite degradation determined by ^{1}H HR MAS MRS [39, 119]. Furthermore, the tissue metabolite concentrations determined in our study are comparable to those previously reported for breast cancer tissue samples stored for five years or less [39, 111]. The integrity of RNA quantified by RIN-values was good (paper III). Altogether, this suggests that the storage procedure maintains the quality of the samples well, and potential gene and metabolite degradations should be minimal.

As ^{1}H HR MAS MRS is a non-destructive method, tissue samples were further used for histological examinations to confirm the tumor cells content (paper I). In paper II and III, imprint cytology was used instead of histological sections to confirm the tumor cell content. Imprint cytology has been found to be a reliable, rapid, and easy complementary examination to histological sectioning [120]. Furthermore, the use of imprint cytology advantageously enabled the tissue samples to be further analyzed for gene and/or protein expressions after ^{1}H HR MAS MRS (paper III).

5.2 Metabolomics analyses

Metabolomics analyses assess the downstream products of gene and protein expressions, and have shown to provide both predictive and prognostic information for several types of cancers [76, 77]. Multivariate and quantitative analyses of the ^{1}H HR MAS MR spectra are of key importance for interpreting findings and validating the results. Therapeutic response is often not associated with the change in one biomarker, but rather with the combined influence of many molecular compounds. Multivariate statistical methods were used to identify patterns of several metabolites simultaneously and to discover metabolic features related to the discrimination between distinct classes. Absolute metabolite quantification has an added value, since the concentrations obtained can be compared to other studies and may be easier to interpret in a clinical setting.

MR metabolite profiles of LABC tumors were related to treatment response and long-term survival by PLS-DA and multilevel PLS-DA multivariate statistical methods. Proper validation of the PLS-DA models is necessary to avoid biased results. In paper I, validation of the multivariate models was performed by full cross-validation. This method is simple and widely used, but is also known to give large variance that may lead to overfitting of data and too optimistic results. However, full cross validation was a convenient choice for this smaller size data set when there were not enough samples for a separate test set. In paper II, validation was performed using randomly selected test sets and permutation testing. To avoid overfitted results, the number of LVs was optimized by cross-validation of the whole data set and the same number of LVs was

used for calculation in all repetitions. It is important to keep in mind that the result of classification models is largely dependent on the validation procedure and also the size of the data set, and that too optimistic results are usually due to inappropriate choice of validation [101].

The study of the metabolic changes in tumors during NAC is important as it can assist in improving treatment of breast cancer patients focusing on personalized treatment strategies. Multilevel PLS-DA is a relatively new method for multivariate investigation of the paired structure within the different subjects [103] and was therefore used in paper II. This method enabled the detection of metabolic changes caused by NAC within each patient by subtracting the between patient variations from the data. Metabolic changes detected by multilevel PLS-DA were significantly better then unpaired PLS-DA, resulting in 87.9% versus 68.9% CV classification accuracy, respectively (paper II). Thus, taking advantage of the multilevel structure in the data set was clearly beneficial.

Metabolites that were found to be important for the multivariate classification models were quantified (paper I and II). To quantify the metabolites in the ^{1}H HR MAS MR spectra, the area of the peaks was determined by curve fitting (paper I and III). Spectral prior knowledge (multiplicity etc) is essential for the quality of the fit. Curve fitting is a proper method for area determination of multiple overlapping signals and is a more accurate and reliable method than peak integration. However, curve fitting by Peakfit can be time-consuming. Automatic model-based curve fitting, such as LCModel [121], has been used for quantification of ^{1}H HR MAS spectra of brain tissue [122, 123]. LCModel facilitates the separation of overlapping signals by fitting the data to a linear combination of the complete spectra from pure metabolite solutions; so called basic sets [123]. Such basic sets have not been generated for ^{1}H HR MAS MRS spectra of breast cancer. Curve fitting using PeakFit can be biased by subjective interpretations. Routines for curve fitting were standardized, and curve fitting was performed by one user (paper I and III). We thus consider the inter-spectral effects on peak area calculations to be small. In paper II, the relative metabolite intensities were found by integrating the peak areas in mean normalized spectra. This approach is useful in terms of confirming the

significant differences in the metabolite levels as observed in the loading plots from multivariate analyses. However, the intensity values are not transferable for comparison with results from other studies.

The electronic external reference method ERETIC used in paper I and III is a promising alternative to the use of internal quantitative references [109-111]. There are no concerns about metabolic activity and visibility when using ERETIC. However, quantification using the ERETIC signal is more sensitive to RF field inhomogeneities of HR MAS probes [124] than chemical internal references such as TSP [111]. The effect of RF field inhomogeneities can be reduced by using an insert which ensure that the sample is within the most homogeneous range of the RF coil, thus minimizing signal loss (paper I-III). A higher relative error for ERETIC (8.4%) compared to TSP (2.7%) has been observed using ^{1}H HR MAS MRS for quantification of creatine in a standard solution [111]. However, ERETIC is still considered as the best approach for quantifying tissue metabolites in ^{1}H HR MAS MRS, as TSP can bind to proteins and other membrane components present in the sample causing an overestimation of metabolite concentrations. Periodical calibration of the ERETIC signal is necessary for ensuring accurate quantification over time [109, 110]. In this thesis, the samples were randomized and analyzed within a short period of time to reduce the effect of calibration and technical artifacts. Pulse length based concentration determination (PULCON) is another external reference technique that uses the principal of reciprocity to correct absolute intensities relative to an external standard spectrum [125, 126]. To our knowledge, no studies using PULCON for quantitative ^{1}H HR MAS MRS have so far been published (Pubmed, Scopus).

Metabolite quantification was related to tissue sample weight, as done in previous studies of ^{1}H HR MAS MRS [39, 111, 127]. ^{1}H HR MAS signals of tumor tissue samples arise from both cancer cells and tumor stroma. The spectra therefore represent the average signal from all the constituents in the sample. In general, this is also the case for other types of molecular profiling analyses, including transcriptomics and genomics.

5.3 MR metabolic profiling and clinical treatment response

The most beneficial use of NAC for LABC patients is still under discussion, and both combinations of different chemotherapies, but also monotherapy, have been suggested [10, 128, 129]. Studies investigating chemoresistance to monotherapy as well as the combination of chemotherapies can help optimize and improve the treatment of breast cancer. The clinical response is also of interest as a surrogate marker for survival. Several studies have shown that pathological complete response (pCR) to NAC, which is obtained in 3-26% of breast cancer patients is associated with improved recurrence-free and overall survival [10, 21]. Thus, an improved understanding of breast tumor molecular characteristics would be essential in the design of personalized cancer therapy, thereby achieving better prognosis.

In this thesis, no valid multivariate classification models for the prediction of clinical response could be generated using pre- or post-treatment spectra (paper I and II). In paper I, metabolic changes in response to NAC were evaluated by comparing quantified individual metabolites obtained pre- and post-treatment, because multilevel PLS-DA was yet not implemented as one of our multivariate analytical tools. In patients with partial response, only GPC was found to be significantly decreased, and a decrease in tCho approached significance. In paper II, all patients experienced significant metabolic changes in response to NAC analyzed by multilevel PLS-DA. However, similar metabolic responses were observed in both patients with partial response and with stable disease. In both groups, treatment led to increased levels of lactate and glycine and decreased levels of GPC, while the levels of PC were markedly decreased for some patients. These metabolic changes were similar to those observed for the entire patient cohort.

The tCho level detected with *in vivo* MRS has been suggested as a biomarker for diagnosis and treatment evaluation of breast cancer [57, 58]. Several studies have found decreased tCho levels in response to NAC to be associated with better clinical response [60, 62, 130-132]. Docetaxel treatment of MCF-7 breast cancer xenografts also resulted in decreased *in vivo* tCho levels and cell density [133]. In another study, *ex vivo* PC

levels were found to decrease after treatment with docetaxel in MCF-7 breast cancer xenograft models [134]. Significantly decreased GPC levels in patients with partial response may suggest a better response to NAC (paper I). Unfortunately, GPC cannot be distinguished from the other choline metabolites by *in vivo* MRS due to low spectral resolution.

The lack of patients with progressive disease and complete response in our analyzed cohort could help explain why no clear metabolic differences in response to NAC between the two response groups were observed (paper I and II). According to the protocol, treatment should be interrupted if/when the patients experienced progressive disease. For patients with complete response, no tumor tissue is left for a post-treatment sample. For these patients, examination of metabolic changes in response to NAC of tissue samples taken at an earlier time point then at final surgery would be of interest. Inclusion of pre-treatment samples of patients with progressive disease and complete response could contribute to reveal clearer differences in metabolite profiles between the clinical response groups that can be used to assist the prediction of clinical response.

The finding that all patients had a similar metabolic response to NAC could be due to the fact that only two patients had an equal or increased tumor size after NAC (paper II). Thus, nearly all patients thus had a reduction in tumor size, although the tumor reduction was small for patients with stable disease. In this thesis, treatment response was defined according to the WHO criteria (Table 1.2), which define stable disease as < 50% reduction to < 25% increase in tumor volume. We can postulate that different response criteria for stable disease (lower threshold for reduction in tumor volume) can affect the findings in paper I and II. The differences in response criteria have shown to influence the prediction of response to NAC by *in vivo* MRI [135].

The fact that all patients had a significant metabolic response to NAC could suggest a difference in metabolite profiles due to differences in sampling procedures for the two time points. However, the tumor samples obtained before NAC were excised during open biopsy surgery, which should be quite comparable to the sampling procedure at final surgery. Moreover, all samples were snap-frozen and stored in the same way.

Thus, we consider the differences in sampling procedures at pre and post-treatment to have small effects on the metabolite profiles.

The metabolic response to NAC is also dependent on the molecular effects of the given chemotherapeutic agents. Anthracyclines, such as doxorubicin and epirubicin, are capable of inducing DNA strand breakage and apoptosis [23-25], while paclitaxel causes mitotic arrest by disturbing the breaking and rearranging of microtubules in the cells [26]. However, the main function of all these drugs is to cause disturbance of the cell cycle and cell death. The metabolic responses to epirubicin and paclitaxel could not be distinguished, which suggests that they have the similar effects on tumor cell metabolism (paper II).

5.4 MR metabolic profiling and long-term survival

The definition of long-term survival is not unified in the literature. A long-term survivor is defined by the American Cancer Society as a person who is still alive 5 years after diagnosis [136]. LABC consist of a heterogeneous group of patients with variable prognosis and with a lower 5-year survival rate than early stage breast cancer [10]. We thus consider the use of the term long-term survival to be appropriate for our studies.

Altered cell metabolism is suggested as a new emerging hallmark of cancer [34]. MR detected metabolite profiles have been found to assist the diagnosis and treatment monitoring of breast cancer [89, 90], and have also been found to correlate to parameters of clinical importance such as tumor grade, axillary lymphatic spread, and hormone receptor status [39, 91, 92]. LABC patients constitute a heterogeneous group with variable prognosis, and it is therefore a challenge to identify valid prognostic factors for this patient group. In addition, prediction of survival in LABC patients receiving NAC is difficult as the treatment will affect the prognostic value of tumor size, grade and lymph node status.

Several studies have shown that a pathological complete response to NAC is associated with improved breast cancer survival [21, 22]. However, approximately 80% of patients will have residual tumor in the breast after NAC [22]. Thus, molecular characterization with identification of new prognostic factors and therapeutic targets for breast cancer would greatly benefit the assessment of prognosis.

In this thesis, the potential of MR metabolomics to predict long-term breast cancer survival was investigated. A summary of the main findings is presented in table 5.1. In paper I, metabolic changes in response to NAC were detected by quantification of individual metabolites. Lactate could not be quantified using the present ^{1}H HR MAS MRS protocol due to heavily overlapping signals from lipids. In paper II, different metabolic responses to NAC were observed in survivors and non-survivors by multilevel PLS-DA.

Prediction of long-term survival was investigated using ^{1}H HR MAS spectra of pre- and post-treatment tumor samples by PLS-DA and metabolite quantification. In paper I, long-term survival could be predicted using pre- and post-treatment spectra. Better classification was obtained post-treatment. In paper II, long-term survival could only be predicted from the post-treatment spectra. These results suggest that the difference between survivors and non-survivors post-treatment is mainly caused by metabolic response to NAC. Thus, the metabolite profiles of post-treatment samples may be a better and more accurate indicator of patient prognosis than pre-treatment samples.

Table 5.1 Summary of metabolite profiles pre- and post-treatment and metabolic changes observed by multivariate analysis and quantification related to long-term survival in paper I and II.

		Paper I		Paper II	
		Multivariate	Quantification	Multivariate	Quantification
Metabolite profiles	Pre-treatment non-survivors *vs* survivors*	⇧ Lac ⇩tCho	No significant values	No significant model	NA
Metabolic changes in response to treatment	Survivors	NA	⇩GPC and Cho	⇩GPC	⇩ Gly, GPC PC, and Cho
	Non-survivors	NA	No significant values	⇧ Lac	⇧ Lac
Metabolite profiles	Post-treatment non-survivors *vs* survivors*	⇧ Lac, Gly, and tCho	No significant values	⇧ Lac, Gly	⇧ Gly

NA, not applicable. *the results describe the metabolite profiles of non-survivors compared to survivors. Metabolite quantification was performed for Lac (not in paper I), Gly, GPC, PC, Cho, and Tau.

In paper I, higher levels of lactate were observed pre- and post-treatment in non-survivors compared to survivors. In paper II, non-survivors had a significant increase in lactate levels resulted in higher lactate levels in non-survivors compared to survivors at post-treatment (paper II). High lactate levels have been found to correlate with increased risk of radiation resistance, high incident of distant metastases and recurrence, and low survival rates in several types of cancers [42-44]. However, molecular mechanisms behind the role of lactate on malignant behavior of cancer cells are not fully understood. Recently, modification of cell energy metabolism has been suggested as an emerging hallmark of cancer [34]. Cancer cells can reprogram their energy metabolism to convert most glucose to lactate regardless of the oxygen supply, a phenomenon known as aerobic glycolysis (the Warburg effect). Aerobic glycolysis is possibly an adaption to intermittent hypoxia; protecting cancer cells from acid-induced toxicity, and facilitating the uptake and incorporation of nutrients needed for cell proliferation [36, 37]. In return, HiF-1 hypoxia induced gene can accentuate glycolysis by activation of multiple enzymes involved in the glycolytic pathway, including LDH,

the enzyme responsible for conversion of pyruvate to lactate [46-49, 51, 52]. Lactate may therefore serve as a maker for both aerobic glycolysis and hypoxic responses in tumors. Increased aerobic glycolysis and tumor hypoxic responses have been shown to facilitate cell proliferation, resistance to apoptosis, angiogenesis, and increased risk of metastasis in cancers [37, 38, 50]. Inhibition of LDH has shown to reduce the glycolytic activity associated with a decrease in tumor proliferation of breast cancer animal models [137]. In breast cancer, HiF-1 expression is highly associated with tumor progression, metastasis, therapy resistance and mortality [138, 139]. Anthracyclines have been shown to inhibit the transcriptional activity of HiF-1 by blocking its binding to DNA [140]. Thus, increased lactate levels observed in non-survivors in response to NAC may also be a marker for chemotherapy resistance in these patients. It has also been suggested that the resulting low pH mediated by high lactate levels may enhance the invasiveness of tumor cells and help them evade tumor-attacking immune cells [141]. This thesis suggests that the increased levels of lactate in response to NAC and higher lactate levels post-treatment observed in non-survivors may reflect enhancement of aerobic glycolytic activity and/or tumor hypoxic responses related to higher tumor malignancy and poor prognosis. Coherently, the glucose levels were increased in survivors but not in non-survivors in response to NAC (paper II). Increased glucose may be indicative of decreased aerobic glycolysis and tumor hypoxic response favorable of long-term breast cancer survival.

In response to NAC, survivors had a significant decrease in glycine levels (paper II). This was reflected in the post-treatment spectra, showing significantly higher levels of glycine in non-survivors compared to survivors. The role of glycine in tumor malignancy is still unclear. Glycine is a downstream product of 3-phosphoglycerate, an intermediate of the glycolysis, but it can also be synthesized from Cho through the glycine-betaine pathway. The biomarker potential of glycine has been studied in human brain tumors, where it was found to positively correlate with tumor grade [40, 41]. Higher levels of glycine have been detected in the more aggressive basal-like breast cancer animal model compared to the luminal-like model [142]. This thesis suggests that the decreased glycine levels detected in survivors in response to NAC can possibly

be caused by altered glycolysis and/or reduced Cho levels associated with reduced tumor malignancy.

In paper I, higher levels of tCho were observed in survivors compared to non-survivors before NAC. In response to NAC, survivors experienced a significant decrease in GPC and Cho measured by quantification. After NAC, lower levels of tCho were observed in survivors compared to non-survivors in the loading plot. However, the differences in tCho levels determined by quantification were not significant, probably due to high interpatient variation. In paper II, survivors experienced a significant decrease in GPC, PC, and Cho levels in response to NAC. The loading plot shows decreased GPC levels in survivors, while the levels of PC were markedly decreased for some patients. Overall, choline metabolite levels decreased in response to treatment in survivors, and appear to be higher in non-survivors compare to survivors post-treatment.

The choline phospholipid metabolism is associated with proliferation, oncogenic signaling, and also tumor hypoxic responses [57]. PC and tCho levels have been shown to increase with increased malignancy in breast cancer cells [66]. CHKA, the enzyme that regulates the phosphorylation of Cho to PC, is known to play an important role in malignant transformation in several types of cancers [143]. Moreover, tCho levels have been shown to correlate with tumor grade and pharmacokinetic parameters of breast tumors, indicating a relation between choline phospholipid metabolism and tumor malignancy, but also angiogenesis [144, 145]. Hypoxic tumor regions co-localize with regions of high tCho, possibly occurring through the up-regulation of CHKA by HiF-1α [65]. This thesis demonstrates that LABC patients with decreased choline phospholipid metabolite levels, in particular GPC levels, in response to NAC have a better survival rate. Furthermore, MR metabolite profiles showing low levels of tCho, lactate, and glycine post-treatment could potentially be predictive of long-term survival. In a recent study, no differences in tumor tCho levels were found between early breast cancer patients with good and poor prognosis [111]. However, the assessment of prognosis in that study was based on the TNM classification system and not survival time.

Five subtypes of breast tumors characterized by gene expression pattern have been identified [146, 147]. A recent study shows the potential of combining transcriptomics and MR metabolic data for a more refine sub-classification of human breast cancers [148]. It is possible that the combined gene expression and MR metabolomics profiles will improve the molecular characteristics of breast cancer and help identify biomarkers, or combination of biomarkers, which in turn can assist treatment stratification and the prediction of patient outcome.

5.5 GDPD5 in the regulation of choline phospholipid metabolism

Both PC and GPC are known to be altered in cancer. In ovarian cancer, relatively low GPC compared to PC is typically observed [149]. In brain tumor, GPC levels is the dominant choline component in low compared to high grade gliomas [122]. Elevated GPC levels have been detected in lung [150] and prostate [151] cancer tissues compared to non-involved tissues. In normal mammary cell cultures, the GPC levels are relatively higher than the PC levels. During malignant transformation PC increases while GPC decreases, resulting in a switch from low to high PC/GPC ratio in cell cultures [66]. In a recent study, higher concentrations of GPC have been observed in basal like (poor prognosis) compared to luminal like (good prognosis) breast cancer xenograft models[142]. While recent research studies have focused on the molecular causes leading to elevated PC levels in cancer, relatively little effort has been made to elucidate the molecular causes behind the changes in GPC levels in cancers. The gene or genes responsible for the changes in GPC levels detected in breast cancer is still unknown.

It is important to understand the molecular causes of the GPC levels detected in breast cancer because it is a part of the *in vivo* tCho signal that is increasingly being used in diagnosis and treatment monitoring of breast cancer patients [58-62]. As shown in paper I and II, targeting the enzymes involved in the regulation of GPC levels in breast cancers may be a good therapeutic target for anti-cancer treatment as significant decreased GPC in response to NAC was observed in patients with long-term survival and better clinical response.

In paper III, the role of GDPD1-5 in the regulation of choline phospholipid metabolism of human breast cancer was studied with combined MRS, gene and protein expressions. Out of five GDPDs tested, GDPD5 was found to be significantly overexpressed in highly malignant ER^- compared to weakly malignant ER^+ human breast cancer cells and breast tumor samples. GDPD5 showed significantly positive correlations with PC, tCho, and PC/GPC levels in human breast tumors, and a trend towards negative correlation with GPC levels. Human breast cancers with choline metabolite profiles consisting of high PC and low GPC levels highly co-expressed GDPD5, CHKA and PLD1, while cancers containing high GPC and relatively low PC levels displayed low co-expression of GDPD5, CHKA, and PLD1. High correlations were found between gene and protein levels of GDPD5 and CHKA.

GDPD5 is a ubiquitously expressed transmembrane protein in human tissues, but has a relatively low expression in kidney and prostate tissues [152]. It has been suggested as a candidate gene for GPC-PDE as down-regulation of GDPD5 efficiently increased GPC levels, while overexpression of GDPD5 contributes to GPC reduction in mouse renal cells [75]. As a candidate gene for GPC-PDE, the GDPD5 expression is expected to inversely correlate with the GPC level. However, the expression of GDPD5 showed only a modest inverse correlation with GPC levels in breast tumors and cells (paper III). This observation suggests the possibility of additional genes responsible for the altered GPC level observed in breast cancer. Nucleotide pyrophosphatases/phosphodiesterases 6 (NPP6) preferably hydrolyze choline-containing phospholipids or phosphodiesters, including GPC [153]. To date, the role of NPP6 is still unknown. The GPC level is also regulated by the gene expression of PLA2 and LYSO-PLA1 [74]. The correlations between these genes and GDPD5 have not been studied.

The strong positive correlations between the expression of GDPD5 and PC, tCho, and PC/GPC levels are probable due to the co-expression of GDPD5, CHKA, and PLD1 observed in breast tumors (paper III). CHKA plays an important role in oncogenic transformation and carcinogenesis in cancers [68, 143, 154, 155]. PLD activity, which degrades PtdCho to Cho, also plays a significant role in oncogenic transformation [156]. Overexpression of CHKA and elevated PC and tCho levels of breast cancer cells have

been associated with increases invasiveness and drug resistance [157]. Treatment with the CHK inhibitor Mn58b has shown to reduce PC levels and inhibit proliferation of cancer cells, and Mn58b therefore appears to have a potential as an anticancer drug [158]. The strong positive correlations between GDPD5, CHKA and PLD1 observed suggest the expression of GDPD5 to be associated with malignancy. Targeting GDPD5 will most likely increase GPC levels. However, accumulation of GPC mediated by GDPD5 may disturb the recycle of Cho essential for renewed synthesis of PtdCho in the cell membrane. More studies are needed to investigate the mechanism and effects of GDPD5 for use as target in cancer treatment.

Overexpression of GDPD5, CHKA, and PLD1 were found in ER^- tumors compared to ER^+ tumors (paper III). ER status is an important factor for the prediction of prognosis and is routinely used for treatment planning in the clinic. Consistent with our results, overexpression of CHKA has been associated with ER^- breast tumors in a previous study [68]. However, ER^- human breast cancer tumors have been related with higher levels of GPC and lower levels of PC compared to ER^+ tumors [92]. The patients included in paper III have been stratified to match the ER status and choline metabolite profiles of the two breast cancer cell lines, MCF-7 and MDB-MB-231, thus a larger cohort is needed to elucidate the relationship between GDPD5 and ER status. This thesis identified GDPD5 as a GPC-PDE gene that likely participates in regulating choline phospholipid metabolism in breast cancer, which possibly occurs in cooperation with CHKA and PLD1.

6 Conclusions and future perspectives

In this thesis, MR metabolic profiling of tumor tissue obtained from LABC patients was used to predict long-term survival and clinical treatment response to NAC by multivariate data analyses and metabolite quantification. Furthermore, the role of GDPDs in choline phospholipid metabolism was investigated.

^{1}H HR MAS MRS analysis of both pre- and post-treatment tumor tissue samples can be valuable as it provides information on metabolic changes in tumors during NAC. This thesis shows that epirubicin and paclitaxel had similar effects on tumor cell metabolism. It would be of interest to examine the metabolic responses in patients receiving other types of single drugs or multidrug treatment to improve the understanding of treatment effects on tumor metabolism.

No clear differences in metabolic responses were detected between patients with partial response and stable disease, thus the prediction of clinical treatment response by MR metabolomics was not successful. However, it is possible that a cohort including also patients with progressive disease would reveal clearer differences in metabolite profiles between the clinical response groups. Also, the chosen response criteria might have contributed to the unsuccessful prediction of clinical treatment response from metabolite profiles. Different response criteria should be tested, in particular pathological measured response. Molecular characterization of tumors may help stratifying patients for individualized treatment, thereby achieving better prognosis.

MR metabolite profiles of LABC patients contain prognostic information that is associated with survival status. In response to NAC, lactate is increased in non-survivors, while survivors experience a decrease in glycine and choline phospholipid

metabolites. These metabolic changes are associated with tumor proliferation, glycolytic activity and hypoxia related to tumor malignancy and prognosis. Metabolite profiles consisting of higher levels of lactate, glycine, and tCho observed in non-survivors post-treatment were shown to be predictive of low breast cancer survival rates. Monitoring metabolic responses to NAC by ^{1}H HR MAS MRS may have the potential to assist the prediction of breast cancer survival and help identify new targets for therapeutic treatment.

The relative fold change in gene expression of GDPD5 was correlated with choline phospholipid metabolite levels and with CHKA and PLD1 expressions. Future studies examining the effect of silencing GDPD5 alone or in combination with CHKA and/or PLD1 in breast cancer cells and xenograft models may provide new information about the potential of GDPD5 as anticancer targets of breast cancer. Studies investigating the correlation between GDPD5 and NPP6, PLA2, and LYSO-PLA1 in choline phospholipid metabolism would be of interest. Overexpression of GDPD5, CHKA, and PLD1 were found in ER^{-} tumors compared to ER^{+} tumors. ER status is associated with treatment response and survival. However, a larger cohort is needed to investigate the correlation between GDPD5 and ER status.

Findings from this thesis show that MR metabolomics can assist the identification of patients at high risk of breast cancer death, and help identify pathways for novel targeted treatment.

References

1. Forouzanfar MH, Foreman KJ, Delossantos AM, Lozano R, Lopez AD, Murray CJ, et al. Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. Lancet. 2011;378(9801):1461-84.
2. Larsen IK, Grimsrud TK, Haldorsen T, Johannesen TB, Johansen A, Langseth H, et al. Cancer in Norway 2009 - Cancer incidence, mortality, survival and prevalence in Norway. Oslo: Cancer Registry of Norway, 2011 June.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: A Cancer Journal for Clinicians. 2011;61(2):69-90.
4. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. American journal of human genetics. 1998;62(3):676-89.
5. Liu C, Zhang H, Shuang C, Lu Y, Jin F, Xu H, et al. Alterations of ER, PR, HER-2/neu, and P53 protein expression in ductal breast carcinomas and clinical implications. Med Oncol. 2010;27(3):747-52.
6. McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. BMJ. 2000;321(7261):624-8.
7. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol. 2001;2(3):133-40.
8. Li CI, Anderson BO, Daling JR, Moe RE. Trends in incidence rates of invasive lobular and ductal breast carcinoma. JAMA : the journal of the American Medical Association. 2003;289(11):1421-4.
9. Tejler G, Norberg B, Dufmats M, Nordenskjöld B. Survival after treatment for breast cancer in a geographically defined population. Brit J Surg. 2004;91(10):1307-12.
10. Mathew J, Asgeirsson KS, Cheung KL, Chan S, Dahda A, Robertson JF. Neoadjuvant chemotherapy for locally advanced breast cancer: a review of the literature and future directions. Eur J Surg Oncol. 2009;35(2):113-22.
11. Franceschini G, Terribile D, Magno S, Fabbri C, D'Alba PF, Chiesa F, et al. Update in the treatment of locally advanced breast cancer: a multidisciplinary approach. Eur Rev Med Pharmacol Sci. 2007;11(5):283-9.
12. AJCC Cancer Staging Manual, 7th edition. American Joint Committee on Cancer, 2009.
13. Chia S, Swain SM, Byrd DR, Mankoff DA. Locally advanced and inflammatory breast cancer. J Clin Oncol. 2008;26(5):786-90.
14. Soerjomataram I, Louwman MW, Ribot JG, Roukema JA, Coebergh JW. An overview of prognostic factors for long-term survivors of breast cancer. Breast Cancer Res and Treat. 2008;107(3):309-30.
15. Roodi N, Bailey LR, Kao WY, Verrier CS, Yee CJ, Dupont WD, et al. Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J Natl Cancer Inst. 1995;87(6):446-51.
16. Liu S, Chia SK, Mehl E, Leung S, Rajput A, Cheang MC, et al. Progesterone receptor is a significant factor associated with clinical outcomes and effect of adjuvant tamoxifen therapy in breast cancer patients. Breast Cancer Res and Treat. 2010;119(1):53-61.
17. Zahnow CA. ErbB receptors and their ligands in the breast. Expert Rev Mol Med. 2006;8(23):1-21.
18. Chang JC. HER2 inhibition: from discovery to clinical practice. Clin Cancer Res. 2007;13(1):1-3.
19. Oakman C, Tenori L, Biganzoli L, Santarpia L, Cappadona S, Luchinat C, et al. Uncovering the metabolomic fingerprint of breast cancer. Int J Biochem Cell B. 2011;43(7):1010-20.
20. De Lena M, Zucali R, Viganotti G, Valagussa P, Bonadonna G. Combined chemotherapy-radiotherapy approach in locally advanced (T3b-T4) breast cancer. Cancer Chemother Pharmacol. 1978;1(1):53-9.
21. Jones RL, Lakhani SR, Ring AE, Ashley S, Walsh G, Smith IE. Pathological complete response and residual DCIS following neoadjuvant chemotherapy for breast carcinoma. Br J Cancer. 2006;94(3):358-62.

22. Kong X, Moran MS, Zhang N, Haffty B, Yang Q. Meta-analysis confirms achieving pathological complete response after neoadjuvant chemotherapy predicts favourable prognosis for breast cancer patients. Eur J Cancer. 2011;In Press.
23. Kiyomiya K, Matsuo S, Kurebe M. Mechanism of specific nuclear transport of adriamycin: the mode of nuclear translocation of adriamycin-proteasome complex. Cancer Res. 2001;61(6):2467-71.
24. Marco A, Arcamone F. DNA complexing antibiotics: daunomycin, adriamycin and their derivatives. Arzneimittelforschung. 1975;25(3):368-74.
25. Perego P, Corna E, De Cesare M, Gatti L, Polizzi D, Pratesi G, et al. Role of apoptosis and apoptosis-related genes in cellular response and antitumor efficacy of anthracyclines. Curr Med Chem. 2001;8(1):31-7.
26. McGrogan BT, Gilmartin B, Carney DN, McCann A. Taxanes, microtubules and chemoresistant breast cancer. Biochim Biophys Acta - Reviews on Cancer. 2008;1785(2):96-132.
27. Gebski V, Lagleva M, Keech A, Simes J, Langlands AO. Survival effects of postmastectomy adjuvant radiation therapy using biologically equivalent doses: a clinical perspective. J Natl Cancer Inst. 2006;98(1):26-38.
28. Osborne CK. Tamoxifen in the treatment of breast cancer. N Engl J Med. 1998;339(22):1609-18.
29. Hackshaw A, Roughton M, Forsyth S, Monson K, Reczko K, Sainsbury R, et al. Long-term benefits of 5 years of tamoxifen: 10-year follow-up of a large randomized trial in women at least 50 years of age with early breast cancer. J Clin Oncol. 2011;29(13):1657-63.
30. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer. 1981;47(1):207-14.
31. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47.
32. Mann RM, Kuhl CK, Kinkel K, Boetes C. Breast MRI: guidelines from the European Society of Breast Imaging. European radiology. 2008;18(7):1307-18.
33. Hanahan D, Weinberg RA. The Hallmarks of Cancer. Cell. 2000;100(1):57-70.
34. Hanahan D, Weinberg Robert A. Hallmarks of Cancer: The Next Generation. Cell. 2011;144(5):646-74.
35. Warburg O. On the origin of cancer cells. Science (New York, NY). 1956;123(3191):309-14.
36. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer. 2004;4(11):891-9.
37. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science (New York, NY). 2009;324(5930):1029-33.
38. Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol. 2011;27:441-64.
39. Sitter B, Lundgren S, Bathen TF, Halgunset J, Fjosne HE, Gribbestad IS. Comparison of HR MAS MR spectroscopic profiles of breast cancer tissue with clinical parameters. NMR Biomed. 2006;19(1):30-40.
40. Davies NP, Wilson M, Natarajan K, Sun Y, MacPherson L, Brundler MA, et al. Non-invasive detection of glycine as a biomarker of malignancy in childhood brain tumours using in-vivo 1H MRS at 1.5 tesla confirmed by ex-vivo high-resolution magic-angle spinning NMR. NMR Biomed. 2010;23(1):80-7.
41. Righi V, Andronesi OC, Mintzopoulos D, Black PM, Tzika AA. High-resolution magic angle spinning magnetic resonance spectroscopy detects glycine as a biomarker in brain tumors. Int J Oncol. 2010;36(2):301-6.
42. Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW, et al. Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. Int J of Radiat Oncol. 2001;51(2):349-53.
43. Walenta S, Wetterling M, Lehrke M, Schwickert G, Sundfor K, Rofstad EK, et al. High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. Cancer Res. 2000;60(4):916-21.
44. Quennet V, Yaromina A, Zips D, Rosner A, Walenta S, Baumann M, et al. Tumor lactate content predicts for response to fractionated irradiation of human squamous cell carcinomas in nude mice. Radiother Oncol. 2006;81(2):130-5.

45. Tessem MB, Swanson MG, Keshari KR, Albers MJ, Joun D, Tabatabai ZL, et al. Evaluation of lactate and alanine as metabolic biomarkers of prostate cancer using 1H HR-MAS spectroscopy of biopsy tissues. Magn Reson Med. 2008;60(3):510-6.
46. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene. 2010;29(5):625-34.
47. Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev. 2009;23(5):537-48.
48. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. 2008;7(1):11-20.
49. Airley RE, Mobasheri A. Hypoxic regulation of glucose transport, anaerobic metabolism and angiogenesis in cancer: novel pathways and targets for anticancer therapeutics. Chemotherapy. 2007;53(4):233-56.
50. Brown N, Bicknell R. Hypoxia and oxidative stress in breast cancer: Oxidative stress - its effects on the growth, metastatic potential and response to therapy of breast cancer. Breast Cancer Res. 2001;3(5):323 - 7.
51. Mathupala S, Colen C, Parajuli P, Sloan A. Lactate and malignant tumors: A therapeutic target at the end stage of glycolysis. J Bioenerg Biomembr. 2007;39(1):73-7.
52. Weidemann A, Johnson RS. Biology of HIF-1alpha. Cell Death Diff. 2008;15(4):621-7.
53. Cuadrado A, Carnero A, Dolfi F, Jimenez B, Lacal JC. Phosphorylcholine: a novel second messenger essential for mitogenic activity of growth factors. Oncogene. 1993;8(11):2959-68.
54. Yoon SJ, Lyoo IK, Kim HJ, Kim T-S, Sung YH, Kim N, et al. Neurochemical Alterations in Methamphetamine-Dependent Patients Treated with Cytidine-5[prime]-Diphosphate Choline: A Longitudinal Proton Magnetic Resonance Spectroscopy Study. Neuropsychopharmacol. 2010;35(5):1165-73.
55. Nakanishi T, Burg MB. Osmoregulation of glycerophosphorylcholine content of mammalian renal cells. Am J Physiol. 1989;257(4 Pt 1):C795-801.
56. Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. Annu Rev Nutr. 2006;26:229-50.
57. Glunde K, Bhujwalla ZM, Ronen SM. Choline metabolism in malignant transformation. Nat Rev Cancer. 2011.
58. Glunde K, Jacobs MA, Bhujwalla ZM. Choline metabolism in cancer: implications for diagnosis and therapy. Expert review of molecular diagnostics. 2006;6(6):821-9.
59. Katz-Brull R, Lavin PT, Lenkinski RE. Clinical utility of proton magnetic resonance spectroscopy in characterizing breast lesions. J Natl Cancer Inst. 2002;94(16):1197-203.
60. Meisamy S, Bolan PJ, Baker EH, Bliss RL, Gulbahce E, Everson LI, et al. Neoadjuvant chemotherapy of locally advanced breast cancer: predicting response with in vivo (1)H MR spectroscopy--a pilot study at 4 T. Radiology. 2004;233(2):424-31.
61. Manton DJ, Chaturvedi A, Hubbard A, Lind MJ, Lowry M, Maraveyas A, et al. Neoadjuvant chemotherapy in breast cancer: early response prediction with quantitative MR imaging and spectroscopy. Br J Cancer. 2006;94(3):427-35.
62. Baek HM, Chen JH, Nalcioglu O, Su MY. Proton MR spectroscopy for monitoring early treatment response of breast cancer to neo-adjuvant chemotherapy. Ann Oncol. 2008;19(5):1022-4.
63. Besterman JM, Duronio V, Cuatrecasas P. Rapid formation of diacylglycerol from phosphatidylcholine: a pathway for generation of a second messenger. Proc Natl Acad Sci. 1986;83(18):6785-9.
64. Bogin L, Papa MZ, Polak-Charcon S, Degani H. TNF-induced modulations of phospholipid metabolism in human breast cancer cells. Biochim Biophys Acta. 1998;1392(2-3):217-32.
65. Glunde K, Shah T, Winnard PT, Jr., Raman V, Takagi T, Vesuna F, et al. Hypoxia regulates choline kinase expression through hypoxia-inducible factor-1 alpha signaling in a human prostate cancer model. Cancer Res. 2008;68(1):172-80.
66. Aboagye EO, Bhujwalla ZM. Malignant Transformation Alters Membrane Choline Phospholipid Metabolism of Human Mammary Epithelial Cells. Cancer Res. 1999;59(1):80-4.
67. Ronen SM, Jackson LE, Beloueche M, Leach MO. Magnetic resonance detects changes in phosphocholine associated with Ras activation and inhibition in NIH 3T3 cells. Br J Cancer. 2001;84(5):691-6.

68. Ramirez de Molina A, Gutierrez R, Ramos MA, Silva JM, Silva J, Bonilla F, et al. Increased choline kinase activity in human breast carcinomas: clinical evidence for a potential novel antitumor strategy. Oncogene. 2002;21(27):4317-22.
69. Hernandez-Alcoceba R, Fernandez F, Lacal JC. In vivo antitumor activity of choline kinase inhibitors: a novel target for anticancer drug discovery. Cancer Res. 1999;59(13):3112-8.
70. Rodriguez-Gonzalez A, Ramirez de Molina A, Fernandez F, Ramos MA, del Carmen Nunez M, Campos J, et al. Inhibition of choline kinase as a specific cytotoxic strategy in oncogene-transformed cells. Oncogene. 2003;22(55):8803-12.
71. Glunde K, Raman V, Mori N, Bhujwalla ZM. RNA interference-mediated choline kinase suppression in breast cancer cells induces differentiation and reduces proliferation. Cancer Res. 2005;65(23):11034-43.
72. Krishnamachary B, Glunde K, Wildes F, Mori N, Takagi T, Raman V, et al. Noninvasive detection of lentiviral-mediated choline kinase targeting in a human breast cancer xenograft. Cancer Res. 2009;69(8):3464-71.
73. Momchilova A, Markovska T, Pankov R. Ha-ras-transformation alters the metabolism of phosphatidylethanolamine and phosphatidylcholine in NIH 3T3 fibroblasts. Cell biology international. 1999;23(9):603-10.
74. Glunde K, Jie C, Bhujwalla ZM. Molecular causes of the aberrant choline phospholipid metabolism in breast cancer. Cancer Res. 2004;64(12):4270-6.
75. Gallazzini M, Ferraris JD, Burg MB. GDPD5 is a glycerophosphocholine phosphodiesterase that osmotically regulates the osmoprotective organic osmolyte GPC. Proc Natl Acad Sci U S A. 2008;105(31):11026-31.
76. Serkova NJ, Glunde K. Metabolomics of cancer. Methods in molecular biology (Clifton, NJ). 2009;520:273-95.
77. Shang Q, Xiang J-F, Tang Y-L. NMR-based metabonomics: a useful platform of oncology research. Bioanalytical Reviews. 2010;1(2):117-40.
78. Nordstrom A, Lewensohn R. Metabolomics: moving to the clinic. J Neuroimmune Pharmacol. 2010;5(1):4-17.
79. Gowda GA, Zhang S, Gu H, Asiago V, Shanaiah N, Raftery D. Metabolomics-based methods for early disease diagnostics. Expert review of molecular diagnostics. 2008;8(5):617-33.
80. Andrew ER. Magic angle spinning. In Encyclopedia of nuclear magnetic resonance. Chichester: Wiley; 1996.
81. Andrew ER, Bradbury A, Eades RG. Nuclear Magnetic Resonance Spectra from a Crystal rotated at High Speed. Nature. 1958;182(4650):1659.
82. Lowe IJ. Free Induction Decays of Rotating Solids. Phys Rev Lett. 1959;2(7):285.
83. Sitter B, Sonnewald U, Spraul M, Fjosne HE, Gribbestad IS. High-resolution magic angle spinning MRS of breast cancer tissue. NMR Biomed. 2002;15(5):327-37.
84. Cheng LL, Ma MJ, Becerra L, Ptak T, Tracey I, Lackner A, et al. Quantitative neuropathology by high resolution magic angle spinning proton magnetic resonance spectroscopy. Proc Natl Acad Sci U S A. 1997;94(12):6408-13.
85. Martinez-Bisbal MC, Marti-Bonmati L, Piquer J, Revert A, Ferrer P, Llacer JL, et al. 1H and 13C HR-MAS spectroscopy of intact biopsy samples ex vivo and in vivo 1H MRS study of human high grade gliomas. NMR Biomed. 2004;17(4):191-205.
86. Cheng LL, Chang IW, Smith BL, Gonzalez RG. Evaluating human breast ductal carcinomas with high-resolution magic-angle spinning proton magnetic resonance spectroscopy. J Magn Reson. 1998;135(1):194-202.
87. Cheng LL, Wu C-l, Smith MR, Gonzalez RG. Non-destructive quantitation of spermine in human prostate tissue samples using HRMAS 1H NMR spectroscopy at 9.4 T. FEBS Lett. 2001;494(1-2):112-6.
88. Mark G. Swanson ASZ, Z. Laura Tabatabai, Jeffry Simko, Samson Jarso, Kayvan R. Keshari, Lars Schmitt, Peter R. Carroll, Katsuto Shinohara, Daniel B. Vigneron, John Kurhanewicz,. Quantitative analysis of prostate metabolites using 1H HR-MAS spectroscopy. Magn Reson Med. 2006;55(6):1257-64.
89. Bathen TF, Sitter B, Sjobakk TE, Tessem MB, Gribbestad IS. Magnetic resonance metabolomics of intact tissue: a biotechnological tool in cancer diagnostics and treatment evaluation. Cancer Res. 2010;70(17):6692-6.

90. Moestue SA, Engebraaten O, Gribbestad IS. Metabolic effects of signal transduction inhibition in cancer assessed by magnetic resonance spectroscopy. Mol Oncol. 2011;5(3):224-41.
91. Bathen TF, Jensen LR, Sitter B, Fjosne HE, Halgunset J, Axelson DE, et al. MR-determined metabolic phenotype of breast cancer in prediction of lymphatic spread, grade, and hormone status. Breast Cancer Res and Treat. 2007;104(2):181-9.
92. Giskeodegard GF, Grinde MT, Sitter B, Axelson DE, Lundgren S, Fjosne HE, et al. Multivariate modeling and prediction of breast cancer prognostic factors using MR metabolomics. J Proteome Res. 2010;9(2):972-9.
93. Kemsley EK, Le Gall G, Dainty JR, Watson AD, Harvey LJ, Tapp HS, et al. Multivariate techniques and their application in nutrition: a metabolomics case study. Brit J Nutr. 2007;98(1):1-14.
94. Liland KH, Almoy T, Mevik BH. Optimal choice of baseline correction for multivariate calibration of spectra. Applied spectroscopy. 2010;64(9):1007-16.
95. Giskeødegård GF, Bloemberg TG, Postma G, Sitter B, Tessem M-B, Gribbestad IS, et al. Alignment of high resolution magic angle spinning magnetic resonance spectra using warping methods. Anal Chim Acta. 2010;683(1):1-11.
96. Wong JW, Cagney G, Cartwright HM. SpecAlign--processing and alignment of mass spectra datasets. Bioinformatics. 2005;21(9):2088-90.
97. Savorani F, Tomasi G, Engelsen SB. icoshift: A versatile tool for the rapid alignment of 1D NMR spectra. J Magn Reson. 2010;202(2):190-202.
98. Nielsen N-PV, Carstensen JM, Smedsgaard J. Aligning of single and multiple wavelength chromatographic profiles for chemometric data analysis using correlation optimised warping. J Chromatogr A. 1998;805(1-2):17-35.
99. Leardi R, Boggia R, Terrile M. Genetic algorithms as a strategy for feature selection. Journal of Chemometrics. 1992;6(5):267-81.
100. Cho H-W, Kim SB, Jeong MK, Park Y, Ziegler TR, Jones DP. Genetic algorithm-based feature selection in high-resolution NMR spectra. Expert Syst Appl. 2008;35(3):967-75.
101. Westerhuis J, Hoefsloot H, Smit S, Vis D, Smilde A, van Velzen E, et al. Assessment of PLSDA cross validation. Metabolomics. 2008;4(1):81-9.
102. Chong I-G, Jun C-H. Performance of some variable selection methods when multicollinearity is present. Chemometr Intell Lab. 2005;78(1-2):103-12.
103. van Velzen EJJ, Westerhuis JA, van Duynhoven JPM, van Dorsten FA, Hoefsloot HCJ, Jacobs DM, et al. Multilevel Data Analysis of a Crossover Designed Human Nutritional Intervention Study. J Proteome Res. 2008;7(10):4483-91.
104. Westerhuis JA, van Velzen EJ, Hoefsloot HC, Smilde AK. Multivariate paired data analysis: multilevel PLSDA versus OPLSDA. Metabolomics. 2010;6(1):119-28.
105. PeakFit Software, http://www.aspiresoftwareintl.com/html/peakfit.html. [01.June 2009].
106. Sitter B, Bathen T, Tessem M, Gribbestad I. High-resolution magic angle spinning (HR MAS) MR spectroscopy in metabolic characterization of human cancer. Prog Nucl Magn Reson Spectrosc. 2009;54(3-4):239-54.
107. Kriat M, Confort-Gouny S, Vion-Dury J, Sciaky M, Viout P, Cozzone PJ. Quantitation of metabolites in human blood serum by proton magnetic resonance spectroscopy. A comparative study of the use of formate and TSP as concentration standards. NMR Biomed. 1992;5(4):179-84.
108. Helms G. The principles of quantification applied to in vivo proton MR spectroscopy. Eur J Radiol. 2008;67(2):218-29.
109. Akoka S, Barantin L, Trierweiler M. Concentration Measurement by Proton NMR Using the ERETIC Method. Anal Chem. 1999;71(13):2554-7.
110. Albers MJ, Butler TN, Rahwa I, Bao N, Keshari KR, Swanson MG, et al. Evaluation of the ERETIC method as an improved quantitative reference for 1H HR-MAS spectroscopy of prostate tissue. Magn Reson Med. 2009;61(3):525-32.
111. Sitter B, Bathen TF, Singstad TE, Fjosne HE, Lundgren S, Halgunset J, et al. Quantification of metabolites in breast cancer patients with different clinical prognosis using HR MAS MR spectroscopy. NMR Biomed. 2010;23(4):424-31.
112. Martinez-Bisbal MC, Monleon D, Assemat O, Piotto M, Piquer J, Llacer JL, et al. Determination of metabolite concentrations in human brain tumour biopsy samples using HR-MAS and ERETIC measurements. NMR Biomed. 2009;22(2):199-206.

113. Aas T, Borresen AL, Geisler S, Smith-Sorensen B, Johnsen H, Varhaug JE, et al. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nat Med. 1996;2(7):811-4.
114. Chrisanthar R, Knappskog S, Løkkevik E, Anker G, Østenstad B, Lundgren S, et al. Predictive and Prognostic Impact of TP53 Mutations and MDM2 Promoter Genotype in Primary Breast Cancer Patients Treated with Epirubicin or Paclitaxel. PloS one. 2011;6(4):e19249.
115. Thorsen T. Occupied and unoccupied nuclear oestradiol receptor in human breast tumours: relation to oestradiol and progesterone cytosol receptors. J Steroid Biochem. 1979;10(6):661-8.
116. Glunde K, Guggino SE, Solaiyappan M, Pathak AP, Ichikawa Y, Bhujwalla ZM. Extracellular acidification alters lysosomal trafficking in human breast cancer cells. Neoplasia (New York, NY). 2003;5(6):533-45.
117. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8.
118. Eilers PHC. Parametric Time Warping. Anal Chem. 2004;76:404-11.
119. Jordan K, He W, Halpern E, Wu C, Cheng L. Evaluation of Tissue Metabolites with High Resolution Magic Angle Spinning MR Spectroscopy Human Prostate Samples After Three-Year Storage at- 80° C. Biomark Insights. 2007;2:147.
120. Mangia A, Chiriatti A, Chiarappa P, Incalza MA, Antonaci G, Pilato B, et al. Touch imprint cytology in tumor tissue banks for the confirmation of neoplastic cellularity and for DNA extraction. Arch Pathol Lab Med. 2008;132(6):974-8.
121. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med. 1993;30(6):672-9.
122. Righi V, Roda JM, Paz J, Mucci A, Tugnoli V, Rodriguez-Tarduchy G, et al. 1H HR-MAS and genomic analysis of human tumor biopsies discriminate between high and low grade astrocytomas. NMR Biomed. 2009;22(6):629-37.
123. Opstad KS, Bell BA, Griffiths JR, Howe FA. Toward accurate quantification of metabolites, lipids, and macromolecules in HRMAS spectra of human brain tumor biopsies using LCModel. Magn Reson Med. 2008;60(5):1237-42.
124. Ziarelli F, Caldarelli S. Solid-state NMR as an analytical tool: Quantitative aspects. Solid State Nuc Magn Reson. 2006;29(1-3):214-8.
125. Wider G, Dreier L. Measuring protein concentrations by NMR spectroscopy. Journal of the American Chemical Society. 2006;128(8):2571-6.
126. Farrant RD, Hollerton JC, Lynn SM, Provera S, Sidebottom PJ, Upton RJ. NMR quantification using an artificial signal. Magn Reson Chem. 2010;48(10):753-62.
127. Gribbestad IS, Sitter B, Lundgren S, Krane J, Axelson D. Metabolite composition in breast tumors examined by proton nuclear magnetic resonance spectroscopy. Anticancer res. 1999;19(3A):1737-46.
128. Joensuu H, Sailas L, Alanko T, Sunela K, Huuhtanen R, Utriainen M, et al. Docetaxel versus docetaxel alternating with gemcitabine as treatments of advanced breast cancer: final analysis of a randomised trial. Ann Oncol. 2010;21(5):968-73.
129. Silver DP, Richardson AL, Eklund AC, Wang ZC, Szallasi Z, Li Q, et al. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. J Clin Oncol. 2010;28(7):1145-53.
130. Baek HM, Chen JH, Nie K, Yu HJ, Bahri S, Mehta RS, et al. Predicting pathologic response to neoadjuvant chemotherapy in breast cancer by using MR imaging and quantitative 1H MR spectroscopy. Radiology. 2009;251(3):653-62.
131. Jagannathan NR, Kumar M, Seenu V, Coshic O, Dwivedi SN, Julka PK, et al. Evaluation of total choline from in-vivo volume localized proton MR spectroscopy and its response to neoadjuvant chemotherapy in locally advanced breast cancer. Brit J Cancer. 2001;84(8):1016-22.
132. Danishad KK, Sharma U, Sah RG, Seenu V, Parshad R, Jagannathan NR. Assessment of therapeutic response of locally advanced breast cancer (LABC) patients undergoing neoadjuvant chemotherapy (NACT) monitored using sequential magnetic resonance spectroscopic imaging (MRSI). NMR Biomed. 2010;23(3):233-41.
133. Huuse EM, Jensen LR, Goa PE, Lundgren S, Anderssen E, Bofin A, et al. Monitoring the Effect of Docetaxel Treatment in MCF7 Xenografts Using Multimodal In Vivo and Ex Vivo Magnetic Resonance Methods, Histopathology, and Gene Expression. Transl Oncol. 2010;3(4):252-63.

134. Jensen LR, Huuse EM, Bathen TF, Goa PE, Bofin AM, Pedersen TB, et al. Assessment of early docetaxel response in an experimental model of human breast cancer using DCE-MRI, ex vivo HR MAS, and in vivo 1H MRS. NMR Biomed. 2010;23(1):56-65.
135. Heldahl MG, Lundgren S, Jensen LR, Gribbestad IS, Bathen TF. Monitoring neoadjuvant chemotherapy in breast cancer patients: Improved MR assessment at 3 T? J Magn Reson Imaging : JMRI. 2011. 34 (3)547-556.
136. Mols F, Vingerhoets AJJM, Coebergh JW, van de Poll-Franse LV. Quality of life among long-term breast cancer survivors: A systematic review. Eur J Cancer. 2005;41(17):2613-9.
137. Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell. 2006;9(6):425-34.
138. Rundqvist H, Johnson RS. Hypoxia and Metastasis in Breast Cancer Diverse Effects of Hypoxia on Tumor Progression. In: Simon MC, editor.: Springer Berlin Heidelberg; 2010. p. 121-39.
139. Milani M, Harris AL. Targeting tumour hypoxia in breast cancer. Eur J Cancer. 2008;44(18):2766-73.
140. Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL. Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. Proc Natl Acad Sci U S A. 2009;106(7):2353-8.
141. Locasale J, Cantley L. Altered metabolism in cancer. BMC Biology. 2010;8(1):88.
142. Moestue SA, Borgan E, Huuse EM, Lindholm EM, Sitter B, Borresen-Dale AL, et al. Distinct choline metabolic profiles are associated with differences in gene expression for basal-like and luminal-like breast cancer xenograft models. BMC cancer. 2010;10:433.
143. Ramirez de Molina A, Banez-Coronel M, Gutierrez R, Rodriguez-Gonzalez A, Olmeda D, Megias D, et al. Choline kinase activation is a critical requirement for the proliferation of primary human mammary epithelial cells and breast tumor progression. Cancer Res. 2004;64(18):6732-9.
144. Baek H-M, Yu HJ, Chen J-H, Nalcioglu O, Su M-Y. Quantitative correlation between 1H MRS and dynamic contrast-enhanced MRI of human breast cancer. Magn Reson Imaging. 2008;26(4):523-31.
145. Chen JH, Mehta RS, Baek HM, Nie K, Liu H, Lin MQ, et al. Clinical characteristics and biomarkers of breast cancer associated with choline concentration measured by 1H MRS. NMR Biomed. 2011;24(3):316-24.
146. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406(6797):747-52.
147. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(19):10869-74.
148. Borgan E, Sitter B, Lingjaerde OC, Johnsen H, Lundgren S, Bathen TF, et al. Merging transcriptomics and metabolomics--advances in breast cancer profiling. BMC cancer. 2010;10:628.
149. Iorio E, Mezzanzanica D, Alberti P, Spadaro F, Ramoni C, D'Ascenzo S, et al. Alterations of choline phospholipid metabolism in ovarian tumor progression. Cancer Res. 2005;65(20):9369-76.
150. Rocha CM, Barros AS, Gil AM, Goodfellow BJ, Humpfer E, Spraul M, et al. Metabolic profiling of human lung cancer tissue by 1H high resolution magic angle spinning (HRMAS) NMR spectroscopy. J Proteome Res. 2010;9(1):319-32.
151. Swanson MG, Keshari KR, Tabatabai ZL, Simko JP, Shinohara K, Carroll PR, et al. Quantification of choline- and ethanolamine-containing metabolites in human prostate tissues using 1H HR-MAS total correlation spectroscopy. Magn Reson Med. 2008;60(1):33-40.
152. Lang Q, Zhang H, Li J, Yin H, Zhang Y, Tang W, et al. Cloning and characterization of a human GDPD domain-containing protein GDPD5. Mol Biol Rep. 2008;35(3):351-9.
153. Sakagami H, Aoki J, Natori Y, Nishikawa K, Kakehi Y, Arai H. Biochemical and molecular characterization of a novel choline-specific glycerophosphodiester phosphodiesterase belonging to the nucleotide pyrophosphatase/phosphodiesterase family. J Biol Chem. 2005;280(24):23084-93.
154. Nakagami K, Uchida T, Ohwada S, Koibuchi Y, Morishita Y. Increased choline kinase activity in 1,2-dimethylhydrazine-induced rat colon cancer. Jpn J Cancer Res. 1999;90(11):1212-7.

155. Ramirez de Molina A, Rodriguez-Gonzalez A, Gutierrez R, Martinez-Pineiro L, Sanchez J, Bonilla F, et al. Overexpression of choline kinase is a frequent feature in human tumor-derived cell lines and in lung, prostate, and colorectal human cancers. Biochem Biophys Res Commun. 2002;296(3):580-3.
156. Foster DA, Xu L. Phospholipase D in cell proliferation and cancer. Mol Cancer Res. 2003;1(11):789-800.
157. Shah T, Wildes F, Penet M-F, Winnard PT, Glunde K, Artemov D, et al. Choline kinase overexpression increases invasiveness and drug resistance of human breast cancer cells. NMR Biomed. 2010;23(6):633-42.
158. Al-Saffar NM, Troy H, Ramirez de Molina A, Jackson LE, Madhu B, Griffiths JR, et al. Noninvasive magnetic resonance spectroscopic pharmacodynamic markers of the choline kinase inhibitor MN58b in human carcinoma models. Cancer Res. 2006;66(1):427-34.

Paper I

Is not included due to copyright

Paper II

Prognostic value of metabolic response in breast cancer patients receiving neoadjuvant chemotherapy

Maria D. Cao*[,1], Guro F. Giskeødegård*[,1], Tone F. Bathen[1,2], Beathe Sitter[1,2], Anna Bofin[3], Per E. Lønning[4,5], Steinar Lundgren[1,6,7], Ingrid S. Gribbestad[1]

* shared first authorship

[1]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU), 7489 Trondheim, Norway

[2]St Olavs University Hospital, Trondheim, Norway

[3]Department of Laboratory Medicine, Children's and Women's Health, NTNU, 7489 Trondheim, Norway

[4]Department of Oncology, Haukeland University Hospital, 5021 Bergen, Norway

[5]Section of Oncology, Institute of medicine, University of Bergen, 5020 Bergen, Norway

[6]Department of Oncology, St. Olavs Hospital, University Hospital of Trondheim, 7006 Trondheim, Norway

[7]Department of Cancer Research and Molecular Medicine, NTNU, 7489 Trondheim, Norway

Corresponding author: Maria D. Cao, Department of Circulation and Medical Imaging, The Faculty of Medicine, NTNU, MTFS, Postboks 8905, N-7489 Trondheim, Norway. Tel.: +47 73598121, fax: +47 73551350, e-mail address: maria.d.cao@ntnu.no.

Abstract

BACKGROUND: Today's clinical diagnostic tools are insufficient for giving accurate prognosis to breast cancer patients. The aim of our study was to examine the tumor metabolic changes in patients with locally advanced breast cancer caused by neoadjuvant chemotherapy (NAC), relating these changes to clinical treatment response and long-term survival.

METHODS: Patients (n=89) participating in a randomized open-label multicenter study were allocated to receive either NAC as epirubicin or paclitaxel monotherapy. Biopsies were excised pre- and post-treatment, and analyzed by high resolution magic angle spinning magnetic resonance spectroscopy (HR MAS MRS). The metabolite profiles were examined by paired and unpaired multivariate methods and findings of important metabolites were confirmed by spectral integration of the metabolite peaks.

RESULTS: All patients had a significant metabolic response to NAC, and pre- and post-treatment spectra could be discriminated with 87.9%/68.9% classification accuracy by paired/unpaired partial least squares discriminant analysis (PLS-DA) ($p<0.001$). Similar metabolic responses were observed for the two chemotherapeutic agents. The metabolic responses were related to patient outcome. Non-survivors (<5 years) had increased tumor levels of lactate ($p=0.004$) after treatment, while survivors (≥5 years) experienced a decrease in the levels of glycine ($p=0.047$) and choline-containing compounds ($p\leq0.013$) and an increase in glucose ($p=0.002$) levels. The metabolic responses could not be related to clinical treatment response.

CONCLUSIONS: The differences in tumor metabolic response to NAC were associated with breast cancer survival, but not to clinical response. Monitoring metabolic responses to NAC by HR MAS MRS may provide information about tumor biology related to individual prognosis.

Background

The prognosis of patients with locally advanced breast cancer varies largely due to the heterogeneity of the disease, and 5-year survival rates from 50-80% have been reported [1]. Neoadjuvant chemotherapy (NAC) has been established as a standard treatment for locally advanced breast cancer, with anthracyclines and taxanes being among the most frequently used agents. NAC is provided to make primarily inoperable tumors resectable, and will also increase the rate of breast-conserving surgery without any significant increase in local or distal recurrence [2, 3]. Studies investigating the metabolic responses and chemoresistance to single or a combination of drugs are important for effective treatment and better patient outcome.

Patients with a pathological complete response (pCR) after NAC have improved outcome compared to patients with residual disease, thus treatment response is a prognostic indicator. However, only ~20% of patients will achieve a pCR to NAC [4]. Other prognostic factors of breast cancer include axillary lymph node status, tumor size, Her-2 overexpression, histopathological grade, and hormone receptor status. The status of Her-2 and hormone receptors is also predictive of treatment response. Identification of other markers for prognosis and treatment response may help stratify patients for better individualized treatment.

Several studies have shown altered metabolism in cancer compared to normal tissue. Elevated levels of total choline-containing compounds (tCho) are frequently observed in cancer, and may serve as magnetic resonance spectroscopy (MRS) markers for malignancy, both *in vivo* and *ex vivo* [5, 6]. The tCho signal constitutes signals from glycerophosphocholine (GPC), phosphocholine (PC) and free choline (Cho) which are involved in phospholipid metabolism through the Kennedy pathway. A decreased level of tCho detected by *in vivo* MRS has been suggested as a possible marker for treatment response [7, 8]. Altered concentrations of other tissue metabolites, such as increased levels of lactate, have also been associated with malignancy [9, 10]. Elevated lactate levels may be related to hypoxia, a common feature of solid tumors where glucose is catabolised to lactate due to the lack of oxygen. Also under conditions with sufficient oxygen levels, cancer cells may convert glucose to lactate, described as the Warburg effect.

High resolution magic angle spinning (HR MAS) MRS is a non-destructive technique providing highly resolved MR spectra of intact tissues with minimal sample preparation. HR MAS MR spectra provide an overview of the different metabolites that are present in a tissue sample, and can give insight into the complex processes leading to cancer and other diseases. More than 30 metabolites have been identified in breast tissue using HR MAS MRS [11]. Systematic studies of the metabolic state of biological systems using multivariate analysis methods are referred to as metabolomics. MR metabolomics studies of breast cancer have revealed correlations between tissue metabolic profiles and clinical prognostic factors such as hormone receptor status, grade and lymphatic spread [12-14]. Long-term survival of breast cancer patients has been successfully predicted from breast cancer tissue using multivariate classification models [15, submitted manuscript Giskeødegård et al]. The purpose of this study was to examine the metabolic changes in breast cancer tissues resulting from treatment with NAC, and to relate these changes to treatment response and long-term survival. This is the first study to investigate the metabolic response of NAC in a large breast cancer cohort using *ex vivo* MRS.

Methods

Patient and tumor characteristics

We examined a subcohort of breast cancer patients (n = 89) from a larger open-label multicenter study where patients were randomly allocated to receive NAC treatment with either anthracycline (epirubicin, 90 mg/m^2) or taxane (paclitaxel, 200 mg/m^2) monotherapy [16]. The patients were given subsequent adjuvant endocrine treatment according to guidelines from the Norwegian Breast Cancer Group. The inclusion criteria and treatment protocol are fully described elsewhere [16]. Briefly, female breast cancer patients at pre/post menopausal age (≤ 70 years) with locally advanced (stage III, $T_{3/4}$ and/or N_2) non-inflammatory breast cancer with or without limited distant metastasis were recruited in the period 1997-2003. The patients were treated every third week for four cycles. Patients showing a non-satisfactory response were assigned to the opposite treatment. From each patient, an incisional biopsy was taken before treatment with NAC and a post-treatment biopsy was excised during surgical removal of the tumor. The biopsies were immediately snap-frozen and stored in liquid nitrogen in a biobank until use. A part of the pre-treatment tumor biopsy was obtained for routine pathological diagnosis and hormone status assignment. Estrogen (ER) and progesterone receptor (PgR) status were determined by immunohistochemical staining (positive ≥ 10% staining cells). The study was approved by The Regional Committee for Medical and Health Research Ethics (Norwegian Health Region III) and informed written consent was obtained from all patients

Response and survival evaluation

Response to treatment was evaluated using the WHO criteria by the UICC system [17]. Treatment response was assessed clinically by comparing caliper measurements prior to NAC treatment and after the last cycle. In the subcohort included in this study, the patients were classified to have either partial response (≥ 50 % reduction in tumor size (the product of the two largest tumor diameters), but not complete response) or stable disease (< 50 % reduction to ≤ 25 % increase in tumor size). Patients deceased within 5 years after diagnosis were classified as non-survivors whereas patients surviving 5 years or more were classified as survivors.

Histopathological examinations

Prior to HR MAS MRS analysis, imprint cytology smears were prepared from the tissue samples and stained with the May-Grünwald-Giemsa stain (Color-Rapid, Med-Kjemi, Norway). Confirmation of tumor cell content was determined microscopically by a cytopathologist.

HR MAS MRS experiments

HR MAS MRS analyses were performed on a Bruker Avance DRX600 spectrometer (Bruker Biospin GmbH, Germany) equipped with a $^{1}H/^{13}C$ MAS probe with gradient. The run order of the samples was randomized (www.random.org) and blindly analyzed during 18 days. Each sample (15.1±2.8 mg) was cut to fit a 30 µl leak-proof disposable insert (Bruker Biospin Corp, USA) and added phosphate buffered saline (PBS, 3 µl) in D_2O containing trimethylsilyl tetradeuteropropionic acid (TSP, 98.2 mM) for chemical shift referencing. Samples were spun at 5 kHz and spectra were recorded within 31 minutes per sample at 4°C to minimize tissue degradation. Spin-echo spectra (cpmgpr; Bruker) were recorded as previously described [14].

Data preprocessing

Twenty eight spectra were excluded from further studies due to low tumor cell content. The resulting data set consisted of 150 spectra from 85 patients (80 pre-treatment and 70 post-treatment spectra). Characteristics of the included patients and tumors are listed in Table 1. The MR spectra were Fourier transformed into 128 K after 0.3 Hz exponential line broadening. Chemical shifts were referenced to the TSP peak at 0 ppm. The spectral region between 4.69-1.45 ppm, excluding the water peak and large lipid residuals, was chosen for analysis. Signals from ethanol pollutions between 3.69-3.57 ppm were removed together with lipid residual signals between 3.01-1.52 ppm. The spectra were baseline corrected using asymmetric least squares [18] with parameters λ = 1e7 and p = 0.0001, and the minimum value of each spectrum was set to zero by subtracting the lowest value. The spectra were normalized to equal total area, and peak aligned using icoshift [19].

Multivariate data analysis

Partial least squares (PLS) analysis is a regression method for analysis of collinear data with numerous variables. The method is based on extraction of underlying structures, or latent variables (LVs), that maximize the covariance between X (the spectra) and a response variable Y [20]. PLS discriminant analysis (PLS-DA) attempts to discriminate between distinct classes. PLS-DA was performed in Matlab R2009a (The Mathworks, Inc., USA) using PLS_Toolbox 6.2.1 (Eigenvector Research, USA). A PLS-DA model was built on mean-centred spectra from randomly chosen training samples (90 % of the patients) and used to predict the status of test samples (the remaining 10 %). This procedure was repeated 20 times and the average classification results were calculated. The number of LVs to use was chosen by cross-validation of the whole data set and used for all repetitions to avoid biased results. The importance of each variable in the loadings of the PLS-DA was evaluated by variable importance in the projection (VIP) scores [21]. The VIP score positively reflects the variable's influence on the classification, and variables with a score greater than one are generally considered important [21, 22]. To evaluate the statistical significance of the classification results, permutation testing was performed [23]. In permutation testing, the class labels are permuted to resemble random classification. It is then possible to examine if the achieved prediction results of the original data set are significantly different than random predictions. The data set with the permuted class label was divided into training and test sets repeated 20 times as described for the original data set, and the average results were calculated. The permutation procedure was repeated 1000 times, and the prediction error of the original data set was compared to the distribution of prediction errors from the permutation. P-values ≤ 0.05 were considered significant.

Multilevel PLS-DA [24, 25] is an extension of ordinary PLS-DA which can be used as a paired analysis for multivariate data. This analysis can only be used when the data has a multilevel structure, i.e. when interventions are evaluated on the same subject. In multilevel PLS-DA, the *between* subject variation is separated from the *within* subject variation. This is useful in metabolic profiling as the variation between subjects, resulting from differences in age, disease state, genetics and other factors, can obscure the metabolic changes caused by the intervention. The between subject variation is described by the average of the two observations from one subject, whereas the within subject variation is described by the net difference between them. Multilevel PLS-DA was used to examine metabolic changes in the

spectra resulting from NAC treatment. The split-up of variation was done using algorithms made available by van Velzen et al [24]. Further PLS-DA classifications of the within subject variation were performed using PLS_Toolbox as described for the unpaired analyses. The net difference of the spectra pre minus post treatment (positively representing the metabolites higher expressed before treatment) is annotated as control, while the net difference post minus pre treatment (positively representing the metabolites higher expressed after treatment) is annotated as treatment. More specifically,

$$\text{control} = \mathbf{A} - \mathbf{B}$$
$$\text{treatment} = \mathbf{B} - \mathbf{A} \qquad \text{(eq. 1)}$$

where the matrix **A** represents pre-treatment spectra, and matrix **B** represents the post-treatment spectra.

Univariate data analysis

To further validate the important metabolites from the PLS-DA models, relative intensities were found by integrating the peak areas of spectra normalized to equal total areas after removal of lipid residuals (Matlab R2009a, The Mathworks, Inc., USA). Normalization of spectra with the lipid residual signals removed will correct for differences in sample size and tumor cell content, as it can be assumed that most of the lipid signals from breast samples do not originate from cancer cells. Group differences were statistically tested by Wilcoxon rank sum tests or Wilcoxon sign rank for paired analyses, and considered significant if the p-values were ≤ 0.05.

Results

Metabolic response to neoadjuvant chemotherapy

All classification results are summarized in Table 2. An unpaired PLS-DA of the pre- and post-treatment spectra of the whole data set showed a significant difference in the metabolite profiles in response to NAC treatment, indicating a metabolic response to NAC in all patients. However, the specificity of the classification was low (57.1%). When comparing the classification errors of PLS-DA and paired multilevel PLS-DA from 20 different test sets, the multilevel PLS-DA with split-up of the variation decreased the classification error significantly (Wilcoxon rank sum test, $p < 0.001$), showing the beneficial effect of the paired analysis. Treatment and control spectra could be separated with a sensitivity and specificity of 87.9%. Figure 1A shows the scores and loadings of the multilevel PLS-DA. Lactate and PC were of high importance for the discrimination according to the VIP scores in the loadings. The levels of lactate and glycine appear to be increased in response to treatment, while the levels of PC are markedly decreased for some patients. In addition, GPC levels were decreased in response to treatment. No clustering according to the given chemotherapeutic agents could be seen in the multilevel PLS-DA score plot (results not shown), thus the metabolic treatment effects of epirubicin and paclitaxel appear to be indistinguishable.

No differences in metabolic response between clinical response groups

The patients were divided into two groups according to their clinical response (partial response or stable disease), and multilevel PLS-DA was performed on each group separately in order to discover potential differences in metabolic treatment response between the groups. Both for patients with partial response and stable disease there was a significant change in the tumor metabolism in response to NAC treatment, and treatment spectra could be discriminated from controls with high sensitivity and specificity ($\geq$ 80.0%). The metabolic response to NAC as observed in the loading plots was similar for both subgroups, resembling the changes observed for the whole data set (results not shown). Thus, no difference in the metabolic response could be detected between patients with stable disease and partial response.

Different metabolic responses correlate with survival

Accordingly the patients were divided into two groups according to their survival status (5-year survivors or non-survivors). Both for survivors and non-survivors there was a clear change in the tumor metabolism in response to NAC treatment (Figure 1B and C), and treatment spectra could be discriminated from controls with high sensitivity and specificity (≥ 82.5%). However, the metabolic treatment response appears to differ between survivors and non-survivors.

The loadings showed unchanged lactate levels in response to treatment in survivors, while lactate increased in non-survivors with high importance for the discrimination according to the VIP scores. This was confirmed by comparison of the relative intensities from metabolite integrals, showing a significant increase in lactate levels in response to treatment in non-survivors ($p = 0.004$) but not in survivors (Table 3).

Glycine appears to be decreased in survivors according to the loadings, and the difference in relative intensities before and after treatment was significant ($p = 0.047$). For non-survivors, the glycine level appears to be high in some samples from both the control and the treatment group; hence the role of glycine in the loading plot is more difficult to interpret. The glycine change from integrated relative intensities was not significant in non-survivors, with a mean value close to zero.

GPC levels were decreased in the loading plot of survivors with VIP scores showing high importance, while changes in GPC levels in non-survivors were less important for the discrimination. Accordingly, the relative intensities of GPC were significantly lower in response to treatment in survivors ($p < 0.001$) but not in non-survivors.

The loadings show decreased levels of PC in response to treatment in both survivors and non-survivors. However, the change in PC relative intensities was only significant for survivors ($p < 0.001$), but not for non-survivors, possibly due to a high standard error.

Relative intensities of Cho levels were significantly decreased in survivors ($p = 0.013$) in response to treatment, but only a trend of decreased Cho levels was seen in non-survivors ($p =$

0.084). In addition, glucose was significantly increased in survivors ($p = 0.002$). Cho and glucose were not protruding in the loadings, possibly due to low intensity values.

As an overall measure of the partly overlapping choline-containing metabolite peaks (GPC, PC, and Cho), the changes in relative intensities of tCho were calculated. Survivors had a significant decrease in tCho levels in response to treatment ($p < 0.001$), while a trend of decreased tCho levels were detected in non-survivors ($p = 0.091$).

Metabolic traits at pre- and post-treatment

A PLS-DA of the post-treatment spectra showed a significant difference in the metabolite profiles of 5-year survivors and non-survivors after treatment with 70.1% correct classification (Table 2). According to the scores and loadings shown in Figure 2, the tumors of non-survivors appear to have more of the metabolites lactate and glycine, and less GPC and taurine than survivors post-treatment. PC appears to be present in high levels in some samples of both survivors and non-survivors. The glycine level was denoted to be of major importance according to the VIP scores, and the relative intensities of glycine were significantly higher in non-survivors compared to survivors post-treatment ($p = 0.033$, Table 4). Similarly, a trend of higher relative intensities of lactate was observed in non-survivors ($p = 0.089$). No significant differences in the relative intensities of taurine and GPC were observed, however differences in the levels of tCho approached significance ($p = 0.075$) with non-survivors having higher relative intensities than survivors post-treatment.

The metabolic differences between survivor and non-survivors were not seen pre-treatment as the multivariate model could not discriminate the two outcome groups (model not valid). None of the metabolites showed significant differences in relative intensities between survivors and non-survivors pre-treatment (Table 4).

No significant differences in the metabolite profiles at pre- or post-treatment were detected between patients with partial responders and stable disease by PLS-DA. Post-treatment spectra from patients treated with Epirubicin and Paclitaxel could not be discriminated by PLS-DA, further confirming the similarity of the metabolic response of the two chemotherapeutic agents that were used in this study.

Discussion

In this study we examined the metabolic effect of NAC treatment in patients with locally advanced breast cancer. By comparing MR spectra of biopsies taken pre- and post-treatment, significant metabolic changes in response to treatment were found both by paired and unpaired multivariate models. The results using paired multilevel PLS-DA were however superior to those of unpaired PLS-DA, thus taking advantage of the multilevel structure in the data set was clearly beneficial.

Epirubicin and Paclitaxel appear to affect the metabolism of the tumor cells in the same manners, as evidenced both by indistinguishable metabolic responses and similar metabolic traits of the post-treatment spectra. Anthracyclines work by interfering with the synthesis and function of DNA, while taxanes stabilize the microtubules; thereby inhibiting cell division [26, 27]. However, both treatments will eventually result in cell death. This might explain why the two agents appear to have similar metabolic responses.

Interestingly all patient in our study cohort showed clear changes in the metabolite profiles in response to treatment, including also patients categorized to have a clinically stable disease. No differences in the metabolic responses of the clinical response groups were detected. However, when examining the metabolic changes in survivors and non-survivors independently, a difference in the metabolic response to NAC was seen. Non-survivors had a significant increase in lactate levels in response to treatment, while survivors showed no change in lactate levels. As a result, a trend of higher levels of lactate was detected in non-survivors compared to survivors post-treatment. Increased lactate levels may be a marker for tumor aggressiveness as high levels of lactate have been correlated with low survival rates, high incident of distant metastasis and recurrence, and increased risk of radiation resistance in several types of cancer [28-30]. Modification of cell energy metabolism is typically observed in malignant tumors and is suggested as an emerging hallmark of cancer [31]. Under normoxic conditions, cancer cells can reprogram their energy metabolism to largely depend on aerobe glycolysis as their primary energy pathway resulting in increased lactate production; the so-called Warburg effect. It is not fully known why cancer cells prefer aerobe glycolysis over complete oxidation as this would produce far more ATP. It has been hypothesized that lactate may enhance the invasiveness of tumor cells and the resulting low pH may help tumor cells evading tumor-attacking immune cells [32].

In addition to aerobe glycoysis, breast cancer cells are often hypoxic due to poor blood supply [33]. It can be assumed that the large tumors of patients with locally advanced breast cancer will be affected by hypoxia. Hypoxia can induce the transcription factor hypoxia inducible factor-1α (HiF-1α), which in turn upregulates multiple genes involved in the glycolytic pathway, angiogenesis, cell proliferation, and other mechanisms [33-35]. Furthermore HIF-1α promotes transcription of lactate dehydrogenase (LDH) and lactate monocarboxylate transporters (MCT), and thus plays an important role in the production and efflux of lactate in cancer cells [36, 37]. Inhibition of LDH by small interfering RNA (siRNA) in mouse breast tumors has been shown to reduce the glycolytic activity associated with a decrease in tumor proliferation and tumorigenic potential [38]. Thus we can suggest that the increased levels of lactate after NAC treatment observed in non-survivors may reflect enhancement of aerobe glycolytic activity and/or hypoxic tumor responses that confer higher tumor malignancy and poor prognosis. In coherence, the glucose levels were increased in response to treatment in survivors but not in non-survivors. Increased glucose may be indicative of decreased aerobe glycolysis and tumor hypoxic response favorable of long term breast cancer survival.

Survivors had a significant decrease in glycine as a response to treatment, while it remained unchanged in non-survivors. This was reflected in the post-treatment spectra, showing significantly lower levels of glycine in survivors. In a previous study, we also found decreased glycine levels after NAC to be associated with long term breast cancer survival [15]. The biological role of glycine in tumor malignancy is still unclear. Several studies have elucidated the biomarker potential of glycine in human brain tumors, where it was found to positively correlate with tumor grade [39, 40]. Higher levels of glycine have also been detected in pre-clinical studies of the more aggressive basal-like breast cancer model compared to the luminal-like model [41]. In patients, high glycine levels detected in malignant breast tumors have been correlated with poor prognosis [42]. Glycine is mainly synthesized from 3-phosphoglycerate, an intermediate of the glycolysis. In addition, glycine can be synthesized from Cho through the glycine-betaine pathway. We can postulate that the decreased glycine levels after NAC treatment detected in survivors are caused by altered glycolysis and/or reduced Cho levels associated with reduced tumor aggressiveness.

A significant decrease of GPC, PC, Cho levels and the combined tCho level was detected in survivors in response to treatment, whereas non-survivors experienced only a trend of

decrease in Cho and tCho levels. As a result, lower tCho levels in survivors compared to non-survivors post-treatment approached significance. In a previous publication, we showed that GPC and Cho concentrations significantly decreased in patients with long-term survival (≥ 5 years), while non-survivors (< 5 years) had no significant changes in choline phospholipid metabolites in response to NAC [15]. Choline phospholipid metabolites are important biological compounds in cell membrane synthesis and turnover. In addition, tCho levels have been associated with increased malignancy and activation of oncogenic signaling in breast cancer cells [43, 44]. Higher tCho concentrations have been detected in high-grade breast tumors and tumors with higher pharmacokinetic parameters measured with dynamic contrast enhanced MR imaging, indicating a correlation between choline phospholipid metabolism and tumor malignancy and angiogenesis [45, 46]. As previously mentioned, cancer cells may undergo adaptive responses to hypoxia by inducing HiF-1α. Increased tCho levels and choline kinase alpha (CHKA) expressions has been detected in prostate cancer cells and xenografts models under hypoxic compared to normoxic conditions [47]. In the same study, the authors found hypoxic tumor regions to be co-localized with regions of high tCho, which possibly occurred through the up-regulation of CHKA by HiF-1α. CHKA is known to play an important role in malignant transformation in several types of cancer [48]. Overexpression of CHKA and elevated PC and tCho levels of breast cancer cells have been associated with increases invasiveness and drug resistance [49]. Decreased choline phospholipid metabolism after NAC treatment may be associated with lower malignancy that potentially can be used as a predictor of breast cancer survival.

The metabolic responses to NAC treatment appear to be similar in patients with partial response and stable disease. None of the patients in this study had a progressive disease, whereas patients with a complete response would not have any tumor tissue left for a post-treatment biopsy. By definition the group with stable disease can have up to 50% reduction in tumor volume, and indeed only two patients in this study had an equal or increased tumor size after NAC. In that respect, almost all patients had a biological effect of the treatment although the tumor reduction was small for patients with a stable disease. It is conceivable that a cohort including also patients with progressive disease would reveal clearer differences in metabolic response between the clinical response groups. It is however noteworthy that all patients in this study in general had a decrease in tCho after NAC, as tCho is suggested as an *in vivo* biomarker for clinical treatment response.

In this patient cohort, the prediction of overall survival was accomplished with 70.1% classification accuracy using post-treatment spectra, but no prognostic information could be extracted from the pre-treatment spectra. This shows that the difference between survivors and non-survivors post-treatment results from a metabolic response to the treatment. The observed higher levels of lactate and glycine in non-survivors compared to survivors support our previous studies postulating high lactate and glycine levels to be predictive of low breast cancer survival rates (< 5 years).

Prediction of survival in patients receiving NAC is challenging. As NAC will downstage and potentially completely remove the disease, standard prognostic indicators such as tumor size and lymph node status are no longer fully applicable after NAC. Several studies have shown that a pathological complete response after NAC is associated with better survival rates [4]. However, approximately 80% of patients will have residual tumor in the breast after treatment [4]. Our study shows that the metabolic response to treatment may be an indicator of patient prognosis.

Conclusions

By comparing HR MAS MR spectra from biopsies excised before and after NAC treatment, we have revealed significant metabolic changes in breast cancer tumors as a response to treatment. Different metabolic responses could be related to patient outcome, but did not separate patients with partial response from those with stable disease. Non-survivors had increased tumor levels of lactate after treatment, while survivors experienced a decrease in the levels of glycine and choline-containing compounds. These differences in tumor response may reflect tumor aggressiveness associated with breast cancer survival. Monitoring metabolic responses to NAC by HR MAS MRS may provide information about tumor biology related to prognosis, and help identify pathways for targeted therapies.

Authors' contributions

MCD carried out the HR MAS MRS and imprint cytology experiments. GFG carried out Matlab programming and preprocessing of the spectral data. MDC and GFG performed the statistical analyses, interpretations of the results and writing of article. IG, TFB, and BS participated in the design of the study, interpretations of the results and writing of article. AB analyzed the imprint cytology samples and helped to draft the manuscript. PEL and SL recruited the patients, collected the tumor biopsies and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements and Funding

This study was supported by the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU), and the Norwegian Research Council grant no. 10324400 (MDC) and 183379 (GFG).

Competing interests

The authors declare that they have no competing interests.

References

1. Mathew J, Asgeirsson KS, Cheung KL, Chan S, Dahda A, Robertson JF: Neoadjuvant chemotherapy for locally advanced breast cancer: a review of the literature and future directions. *European Journal of Surgical Oncology* 2009, 35:113-122.
2. Makhoul I, Kiwan E: Neoadjuvant systemic treatment of breast cancer. *Journal of Surgical Oncology* 2011, 103:348-357.
3. Makris A, Powles TJ, Ashley SE, Chang J, Hickish T, Tidy VA, Nash AG, Ford HT: A reduction in the requirements for mastectomy in a randomized trial of neoadjuvant chemoendocrine therapy in primary breast cancer. *Annals of Oncology* 1998, 9:1179-1184.
4. Kong X, Moran MS, Zhang N, Haffty B, Yang Q: Meta-analysis confirms achieving pathological complete response after neoadjuvant chemotherapy predicts favourable prognosis for breast cancer patients. *European Journal of Cancer* 2011, In Press, Corrected Proof.
5. Ackerstaff E, Glunde K, Bhujwalla ZM: Choline phospholipid metabolism: a target in cancer cells? *Journal of Cellular Biochemistry* 2003, 90:525-533.
6. Katz-Brull R, Lavin PT, Lenkinski RE: Clinical Utility of Proton Magnetic Resonance Spectroscopy in Characterizing Breast Lesions. *Journal of the National Cancer Institute* 2002, 94:1197-1203.
7. Baek H-M, Chen J-H, Nalcioglu O, Su M-Y: Proton MR spectroscopy for monitoring early treatment response of breast cancer to neo-adjuvant chemotherapy. *Annals of Oncology* 2008, 19:1022-1024.
8. Jagannathan NR, Kumar M, Seenu V, Coshic O, Dwivedi SN, Julka PK, Srivastava A, Rath GK: Evaluation of total choline from in-vivo volume localized proton MR spectroscopy and its response to neoadjuvant chemotherapy in locally advanced breast cancer. *British Journal of Cancer* 2001, 84:1016-1022.
9. Cheng LL, Chang IW, Smith BL, Gonzalez RG: Evaluating human breast ductal carcinomas with high-resolution magic-angle spinning proton magnetic resonance spectroscopy. *Journal of Magnetic Resonance* 1998, 135:194-202.
10. Tessem MB, Swanson MG, Keshari KR, Albers MJ, Joun D, Tabatabai ZL, Simko JP, Shinohara K, Nelson SJ, Vigneron DB, et al.: Evaluation of lactate and alanine as metabolic biomarkers of prostate cancer using 1H HR-MAS spectroscopy of biopsy tissues. *Magnetic Resonance in Medicine* 2008, 60:510-516.
11. Sitter B, Sonnewald U, Spraul M, Fjosne HE, Gribbestad IS: High-resolution magic angle spinning MRS of breast cancer tissue. *NMR in Biomedicine* 2002, 15:327-337.
12. Bathen TF, Jensen LR, Sitter B, Fjosne HE, Halgunset J, Axelson DE, Gribbestad IS, Lundgren S: MR-determined metabolic phenotype of breast cancer in prediction of lymphatic spread, grade, and hormone status. *Breast Cancer Research and Treatment* 2007, 104:181-189.
13. Giskeødegård GF, Grinde MT, Sitter B, Axelson DE, Lundgren S, Fjøsne HE, Dahl S, Gribbestad IS, Bathen TF: Multivariate Modeling and Prediction of Breast Cancer Prognostic Factors Using MR Metabolomics. *J Proteome Res* 2010, 9:972-979.

14. Sitter B, Lundgren S, Bathen TF, Halgunset J, Fjosne HE, Gribbestad IS: Comparison of HR MAS MR spectroscopic profiles of breast cancer tissue with clinical parameters. *NMR in Biomedicine* 2006, 19:30-40.
15. Cao MD, Sitter B, Bathen TF, Bofin A, Lonning PE, Lundgren S, Gribbestad IS: Predicting long-term survival and treatment response in breast cancer patients receiving neoadjuvant chemotherapy by MR metabolic profiling. *NMR Biomed* 2011.
16. Chrisanthar R, Knappskog S, Løkkevik E, Anker G, Østenstad B, Lundgren S, Risberg T, Mjaaland I, Skjønsberg G, Aas T, et al.: Predictive and Prognostic Impact of TP53 Mutations and MDM2 Promoter Genotype in Primary Breast Cancer Patients Treated with Epirubicin or Paclitaxel. *PLoS ONE* 2011, 6:e19249.
17. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD: Assessment of response to therapy in advanced breast cancer. *Cancer* 1977, 39:1289-1294.
18. Eilers PHC: Parametric Time Warping. *Analytical Chemistry* 2004, 76:404-411.
19. Savorani F, Tomasi G, Engelsen SB: icoshift: A versatile tool for the rapid alignment of 1D NMR spectra. *J Magn Reson* 2010, 202:190-202.
20. Wold S, Sjöström M, Eriksson L: PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems* 2001, 58:109-130.
21. Chong I-G, Jun C-H: Performance of some variable selection methods when multicollinearity is present. *Chemometrics and Intelligent Laboratory Systems* 2005, 78:103-112.
22. Chen J, Wang W, Lv S, Yin P, Zhao X, Lu X, Zhang F, Xu G: Metabonomics study of liver cancer based on ultra performance liquid chromatography coupled to mass spectrometry with HILIC and RPLC separations. *Analytica Chimica Acta* 2009, 650:3-9.
23. Westerhuis J, Hoefsloot H, Smit S, Vis D, Smilde A, van Velzen E, van Duijnhoven J, van Dorsten F: Assessment of PLSDA cross validation. *Metabolomics* 2008, 4:81-89.
24. van Velzen EJJ, Westerhuis JA, van Duynhoven JPM, van Dorsten FA, Hoefsloot HCJ, Jacobs DM, Smit S, Draijer R, Kroner CI, Smilde AK: Multilevel Data Analysis of a Crossover Designed Human Nutritional Intervention Study. *Journal of Proteome Research* 2008, 7:4483-4491.
25. Westerhuis JA, van Velzen EJ, Hoefsloot HC, Smilde AK: Multivariate paired data analysis: multilevel PLSDA versus OPLSDA. *Metabolomics* 2010, 6:119-128.
26. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L: Anthracyclines: Molecular Advances and Pharmacologic Developments in Antitumor Activity and Cardiotoxicity. *Pharmacological Reviews* 2004, 56:185-229.
27. McGrogan BT, Gilmartin B, Carney DN, McCann A: Taxanes, microtubules and chemoresistant breast cancer. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 2008, 1785:96-132.
28. Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW, Mueller-Klieser W: Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. *International Journal of Radiation Oncology, Biology, Physics* 2001, 51:349-353.
29. Walenta S, Wetterling M, Lehrke M, Schwickert G, Sundfør K, Rofstad EK, Mueller-Klieser W: High Lactate Levels Predict Likelihood of Metastases, Tumor Recurrence, and Restricted Patient Survival in Human Cervical Cancers. *Cancer Res* 2000, 60:916-921.

30. Quennet V, Yaromina A, Zips D, Rosner A, Walenta S, Baumann M, Mueller-Klieser W: Tumor lactate content predicts for response to fractionated irradiation of human squamous cell carcinomas in nude mice. *Radiotherapy and Oncology* 2006, 81:130-135.
31. Hanahan D, Weinberg Robert A: Hallmarks of Cancer: The Next Generation. *Cell* 2011, 144:646-674.
32. Locasale J, Cantley L: Altered metabolism in cancer. *BMC Biology* 2010, 8:88.
33. Brown N, Bicknell R: Hypoxia and oxidative stress in breast cancer: Oxidative stress - its effects on the growth, metastatic potential and response to therapy of breast cancer. *Breast Cancer Res* 2001, 3:323 - 327.
34. Vander Heiden MG, Cantley LC, Thompson CB: Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science* 2009, 324:1029-1033.
35. Duffy M, Maguire T, Hill A, McDermott E, O'Higgins N: Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. *Breast Cancer Res* 2000, 2:252 - 257.
36. Mathupala S, Colen C, Parajuli P, Sloan A: Lactate and malignant tumors: A therapeutic target at the end stage of glycolysis. *Journal of Bioenergetics and Biomembranes* 2007, 39:73-77.
37. Weidemann A, Johnson RS: Biology of HIF-1alpha. *Cell Death and Differentiation* 2008, 15:621-627.
38. Fantin VR, St-Pierre J, Leder P: Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* 2006, 9:425-434.
39. Davies NP, Wilson M, Natarajan K, Sun Y, MacPherson L, Brundler MA, Arvanitis TN, Grundy RG, Peet AC: Non-invasive detection of glycine as a biomarker of malignancy in childhood brain tumours using in-vivo1H MRS at 1.5 Tesla confirmed by ex-vivo high-resolution magic-angle spinning NMR. *NMR Biomed* 2010, 23:80-87.
40. Righi V, Andronesi OC, Mintzopoulos D, Black PM, Tzika AA: High-resolution magic angle spinning magnetic resonance spectroscopy detects glycine as a biomarker in brain tumors. *International Journal of Oncology* 2010, 36:301-306.
41. Moestue S, Borgan E, Huuse E, Lindholm E, Sitter B, Børresen-Dale A, Engebraaten O, Mælandsmo G, Gribbestad I: Distinct choline metabolic profiles are associated with differences in gene expression for basal-like and luminal-like breast cancer xenograft models. *BMC cancer* 2010, 10:433.
42. Sitter B, Bathen TF, Singstad TE, Fjøsne HE, Lundgren S, Halgunset J, Gribbestad IS: Quantification of metabolites in breast cancer patients with different clinical prognosis using HR MAS MR spectroscopy. *NMR Biomed* 2010, 23:424-431.
43. Aboagye EO, Bhujwalla ZM: Malignant Transformation Alters Membrane Choline Phospholipid Metabolism of Human Mammary Epithelial Cells. *Cancer Research* 1999, 59:80-84.
44. Ronen SM, Jackson LE, Beloueche M, Leach MO: Magnetic resonance detects changes in phosphocholine associated with Ras activation and inhibition in NIH 3T3 cells. *Br J Cancer* 2001, 84:691-696.
45. Baek H-M, Yu HJ, Chen J-H, Nalcioglu O, Su M-Y: Quantitative correlation between 1H MRS and dynamic contrast-enhanced MRI of human breast cancer. *Magnetic Resonance Imaging* 2008, 26:523-531.

46. Chen JH, Mehta RS, Baek HM, Nie K, Liu H, Lin MQ, Yu HJ, Nalcioglu O, Su MY: Clinical characteristics and biomarkers of breast cancer associated with choline concentration measured by 1H MRS. *NMR in Biomedicine* 2011, 24:316-324.
47. Glunde K, Shah T, Winnard PT, Raman V, Takagi T, Vesuna F, Artemov D, Bhujwalla ZM: Hypoxia Regulates Choline Kinase Expression through Hypoxia-Inducible Factor-1α Signaling in a Human Prostate Cancer Model. *Cancer Res* 2008, 68:172-180.
48. de Molina AR, Báñez-Coronel M, Gutiérrez R, Rodríguez-González A, Olmeda D, Megías D, Lacal JC: Choline Kinase Activation Is a Critical Requirement for the Proliferation of Primary Human Mammary Epithelial Cells and Breast Tumor Progression. *Cancer Research* 2004, 64:6732-6739.
49. Shah T, Wildes F, Penet M-F, Winnard PT, Glunde K, Artemov D, Ackerstaff E, Gimi B, Kakkad S, Raman V, Bhujwalla ZM: Choline kinase overexpression increases invasiveness and drug resistance of human breast cancer cells. *NMR Biomed* 2010, 23:633-642.

Table 1 Patient and tumor characteristics

		Survivors (n=60)	**Non-survivors (n=23)**	**NA* (n=2)**
Mean age (±SD)	years	51.1±10.6	49.3±8.3	46.4±2.6
Mean tumor dimensions (mean ±SD)	mm	67.9x67.9 ±18.0x19.8	78.6x77.3 ±22.0x24.0	65.0x51.5 ±7.1x26.2
NAC treatment	Epirubicin	25	8	-
	Paclitaxel	23	5	2
	Both[1]	12	10	-
Treatment response	Partial response	40	11	1
	Stable disease	20	12	1
AJCC	IIB	21	7	1
	IIIA	25	11	1
	IIIB	12	2	-
	IV	2	3	-
ER status	+	41	7	2
	-	19	15	-
	unknown	-	1	-
PgR status	+	32	14	2
	-	28	18	-
	unknown	-	1	-
Nodes	+	32	14	1
	-	28	9	1
Metastasis	+	2	2	0
	-	58	21	2

*NA, not applicable - One patient without following up and one patient dead by other causes; AJCC, American Joint Committee on Cancer; Both[1], sequential treatment with Epirubicin and Paclitaxel

Table 2 Classification results from PLS-DA and multilevel PLS-DA

				Class. model	No. of LVs	Variance X/Y (%)	Sensitivity/ Specificity (%)	Class. accuracy (%)	Permutation p-value
Paired data	All samples	Control vs Treatment	(n=65p)	Multilevel PLS-DA	2	58.9/50.9	87.9/87.9	87.9	<0.001
	Partial response	Control vs Treatment	(n=37p)	Multilevel PLS-DA	2	55.7/61.7	80.0/80.0	80.0	<0.001
	Stable disease	Control vs Treatment	(n=28p)	Multilevel PLS-DA	2	63.2/61.3	88.7/88.7	88.7	<0.001
	Survivors	Control vs Treatment	(n=44p)	Multilevel PLS-DA	2	60.0/50.5	83.0/83.0	83.0	<0.001
	Non-survivors	Control vs Treatment	(n=19p)	Multilevel PLS-DA	2	70.6/63.9	82.5/82.5	82.5	0.006
Unpaired data	All samples	Pre- vs Post-treatment	(n=65p)	PLD-DA	2	50.3/29.2	80.7/57.1	68.9	<0.001
	Pre-treatment	Partial response vs Stable disease	(n=48) (n=32)	PLS-DA	2	51.5/19.3	56.7/60.2	58.4	0.231
	Post-treatment	Partial response vs Stable disease	(n=41) (n=29)	PLS-DA	NaN	-	-	-	-
	Pre-treatment	Survivors vs Non-survivors	(n=57) (n=20)	PLS-DA	NaN	-	-	-	-
	Post-treatment	Survivors vs Non-survivors	(n=47) (n=21)	PLS-DA	3	62.8/32.1	58.4/75.3	70.1	0.009
	Post-treatment	Epirubicin vs Paclitaxel	(n=29) (n=23)	PLS-DA	2	45.7/26.8	45.0/48.3	46.5	0.245

The sensitivity is for detecting a treatment/stable disease/non-survivor/Paclitaxel spectrum; Variance X/Y, amount of variance from X/Y explained by the model; NaN, no valid model; p, pairs.

Table 3 Changes in relative intensities of metabolites in response to NAC

		Survivors		Non-survivors	
Metabolite	**ppm**	**Mean ± SE**	**p-value**	**Mean ± SE**	**p-value**
Lactate	4.08-4.13	2.8 ± 15.3	0.815	97.1 ± 26.4	0.004**
Glycine	3.54-3.56	-19.6 ± 8.0	0.047*	0.8 ± 14.3	0.601
GPC	3.22-3.24	-59.6 ± 14.6	< 0.001**	-11.8 ± 15.7	0.469
PC	3.21-3.22	-95.4 ± 24.3	< 0.001**	-67.4 ± 44.2	0.227
Cho	3.20-3.21	-16.6 ± 6.3	0.013*	-17.8 ± 6.3	0.084
tCho	3.20-3.24	-167.2 ± 36.7	< 0.001**	-95.3 ± 57.5	0.091
Taurine	3.40-3.43	-0.8 ± 13.2	0.861	6.9 ± 12.5	0.398
β-Glucose	4.61-4.64	17.8 ± 5.1	0.002**	-0.2 ± 8.8	0.841

The values (post- minus pre-treatment) of relative intensities are integrated peak areas from spectra normalized to equal total areas. Wilcoxon sign rank tests were used for paired statistical analyses. *$p < 0.05$, **$p < 0.01$.

Table 4 Relative intensities of metabolites at pre- and post-treatment

Metabolites	ppm	Pre-treatment (mean ± SE)			Post-treatment (mean ± SE)		
		Survivors	Non-survivors	p-value	Survivors	Non-survivors	p-value
Lactate	4.08-4.13	185.8 ± 11.4	164.6 ± 13.8	0.534	196.2 ± 12.7	250.6 ± 26.0	0.089
Glycine	3.54-3.56	115.0 ± 6.4	120.8 ± 11.0	0.542	91.2 ± 4.1	111.4 ± 8.4	0.033*
GPC	3.22-3.24	167.3 ± 12.4	170.1 ± 23.9	0.949	115.6 ± 6.7	153.9 ± 24.1	0.144
PC	3.21-3.22	247.9 ± 17.0	285.4 ± 34.9	0.338	158.0 ± 16.1	205.5 ± 28.1	0.105
Cho	3.20-3.21	95.4 ± 3.9	108.7 ± 8.3	0.225	79.5 ± 3.7	85.9 ± 6.2	0.276
tCho	3.20-3.24	498.3 ± 26.4	551.3 ± 46.8	0.253	344.9 ± 22.3	434.6 ± 44.6	0.075
Taurine	3.40-3.43	242.6 ± 9.6	222.8 ± 12.4	0.393	248.0 ± 8.1	222.3 ± 9.3	0.144
β-Glucose	4.61-4.64	48.4 ± 3.3	49.3 ± 6.0	1.000	63.6 ± 4.3	53.5 ± 6.6	0.172

The values of relative intensities are integrated peak areas from spectra normalized to equal total areas. Wilcoxon rank sum tests were used for statistical analyses. $^{*}p < 0.05$, $^{**}p < 0.01$.

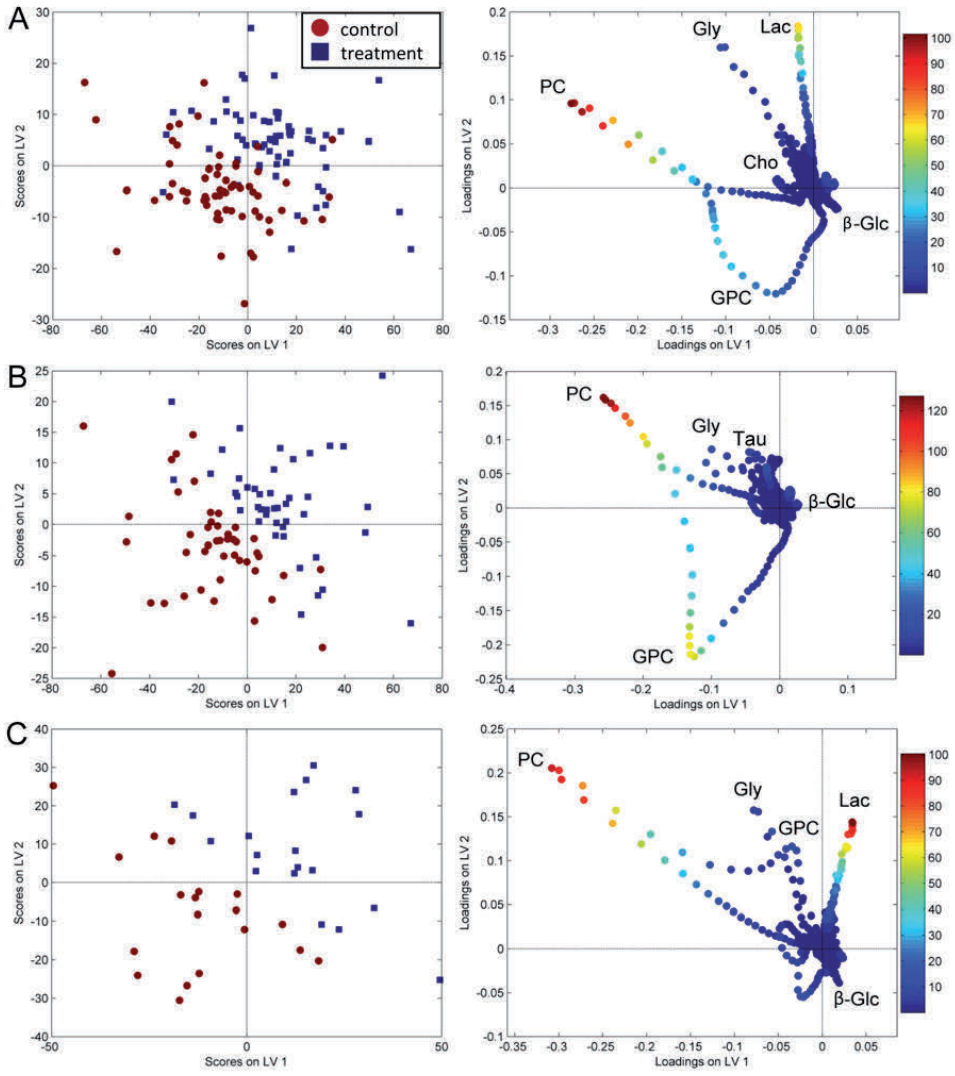

Figure 1 Scores and loadings from multilevel PLS-DA. Separation of treatment and control spectra based on (A) the whole data set, (B) 5-year survivors, and (C) non-survivors. The variables in the loadings are colored according to VIP scores, indicating the importance of each variable in the discrimination. The control spectra equal the difference between pre- and post-treatment spectra, while the treatment spectra equal the post-pre treatment difference. Lac, lactate; Gly, glycine; β-Glc, β-glucose.

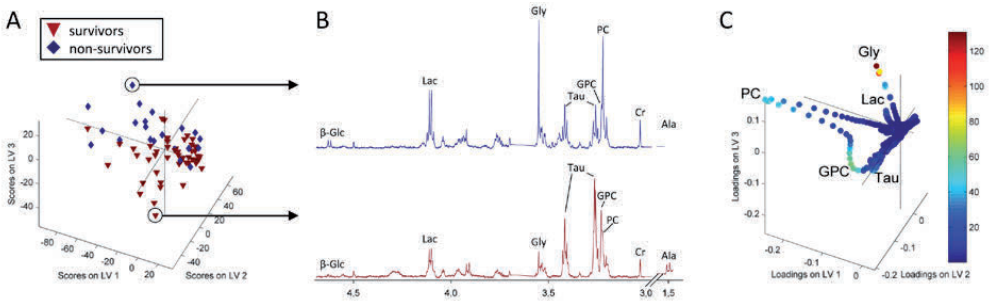

Figure 2 PLS-DA of the MR spectra from biopsies excised post-treatment. (A) A score plot separating survivors and non-survivors, (B) Representative spectra showing the metabolic differences of the tumors of survivors and non-survivors. (C) The loadings of the PLS-DA model with variables colored according to the VIP scores. β-Glc, β-glucose; Lac, lactate, Gly, glycine; Cr, creatine; Ala, alanine

Paper III

Is not included due to copyright

Dissertations at the Faculty of Medicine, NTNU

1977

1. Knut Joachim Berg: EFFECT OF ACETYLSALICYLIC ACID ON RENAL FUNCTION
2. Karl Erik Viken and Arne Ødegaard: STUDIES ON HUMAN MONOCYTES CULTURED *IN VITRO*

1978

3. Karel Bjørn Cyvin: CONGENITAL DISLOCATION OF THE HIP JOINT.
4. Alf O. Brubakk: METHODS FOR STUDYING FLOW DYNAMICS IN THE LEFT VENTRICLE AND THE AORTA IN MAN.

1979

5. Geirmund Unsgaard: CYTOSTATIC AND IMMUNOREGULATORY ABILITIES OF HUMAN BLOOD MONOCYTES CULTURED IN VITRO

1980

6. Størker Jørstad: URAEMIC TOXINS
7. Arne Olav Jenssen: SOME RHEOLOGICAL, CHEMICAL AND STRUCTURAL PROPERTIES OF MUCOID SPUTUM FROM PATIENTS WITH CHRONIC OBSTRUCTIVE BRONCHITIS

1981

8. Jens Hammerstrøm: CYTOSTATIC AND CYTOLYTIC ACTIVITY OF HUMAN MONOCYTES AND EFFUSION MACROPHAGES AGAINST TUMOR CELLS *IN VITRO*

1983

9. Tore Syversen: EFFECTS OF METHYLMERCURY ON RAT BRAIN PROTEIN.
10. Torbjørn Iversen: SQUAMOUS CELL CARCINOMA OF THE VULVA.

1984

11. Tor-Erik Widerøe: ASPECTS OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS.
12. Anton Hole: ALTERATIONS OF MONOCYTE AND LYMPHOCYTE FUNCTIONS IN REALTION TO SURGERY UNDER EPIDURAL OR GENERAL ANAESTHESIA.
13. Terje Terjesen: FRACTURE HEALING AND STRESS-PROTECTION AFTER METAL PLATE FIXATION AND EXTERNAL FIXATION.
14. Carsten Saunte: CLUSTER HEADACHE SYNDROME.
15. Inggard Lereim: TRAFFIC ACCIDENTS AND THEIR CONSEQUENCES.
16. Bjørn Magne Eggen: STUDIES IN CYTOTOXICITY IN HUMAN ADHERENT MONONUCLEAR BLOOD CELLS.
17. Trond Haug: FACTORS REGULATING BEHAVIORAL EFFECTS OG DRUGS.

1985

18. Sven Erik Gisvold: RESUSCITATION AFTER COMPLETE GLOBAL BRAIN ISCHEMIA.
19. Terje Espevik: THE CYTOSKELETON OF HUMAN MONOCYTES.
20. Lars Bevanger: STUDIES OF THE Ibc (c) PROTEIN ANTIGENS OF GROUP B STREPTOCOCCI.
21. Ole-Jan Iversen: RETROVIRUS-LIKE PARTICLES IN THE PATHOGENESIS OF PSORIASIS.
22. Lasse Eriksen: EVALUATION AND TREATMENT OF ALCOHOL DEPENDENT BEHAVIOUR.
23. Per I. Lundmo: ANDROGEN METABOLISM IN THE PROSTATE.

1986

24. Dagfinn Berntzen: ANALYSIS AND MANAGEMENT OF EXPERIMENTAL AND CLINICAL PAIN.
25. Odd Arnold Kildahl-Andersen: PRODUCTION AND CHARACTERIZATION OF MONOCYTE-DERIVED CYTOTOXIN AND ITS ROLE IN MONOCYTE-MEDIATED CYTOTOXICITY.
26. Ola Dale: VOLATILE ANAESTHETICS.

1987

27. Per Martin Kleveland: STUDIES ON GASTRIN.
28. Audun N. Øksendal: THE CALCIUM PARADOX AND THE HEART.
29. Vilhjalmur R. Finsen: HIP FRACTURES

1988

30. Rigmor Austgulen: TUMOR NECROSIS FACTOR: A MONOCYTE-DERIVED REGULATOR OF CELLULAR GROWTH.
31. Tom-Harald Edna: HEAD INJURIES ADMITTED TO HOSPITAL.
32. Joseph D. Borsi: NEW ASPECTS OF THE CLINICAL PHARMACOKINETICS OF METHOTREXATE.
33. Olav F. M. Sellevold: GLUCOCORTICOIDS IN MYOCARDIAL PROTECTION.
34. Terje Skjærpe: NONINVASIVE QUANTITATION OF GLOBAL PARAMETERS ON LEFT VENTRICULAR FUNCTION: THE SYSTOLIC PULMONARY ARTERY PRESSURE AND CARDIAC OUTPUT.
35. Eyvind Rødahl: STUDIES OF IMMUNE COMPLEXES AND RETROVIRUS-LIKE ANTIGENS IN PATIENTS WITH ANKYLOSING SPONDYLITIS.
36. Ketil Thorstensen: STUDIES ON THE MECHANISMS OF CELLULAR UPTAKE OF IRON FROM TRANSFERRIN.
37. Anna Midelfart: STUDIES OF THE MECHANISMS OF ION AND FLUID TRANSPORT IN THE BOVINE CORNEA.
38. Eirik Helseth: GROWTH AND PLASMINOGEN ACTIVATOR ACTIVITY OF HUMAN GLIOMAS AND BRAIN METASTASES - WITH SPECIAL REFERENCE TO TRANSFORMING GROWTH FACTOR BETA AND THE EPIDERMAL GROWTH FACTOR RECEPTOR.
39. Petter C. Borchgrevink: MAGNESIUM AND THE ISCHEMIC HEART.
40. Kjell-Arne Rein: THE EFFECT OF EXTRACORPOREAL CIRCULATION ON SUBCUTANEOUS TRANSCAPILLARY FLUID BALANCE.
41. Arne Kristian Sandvik: RAT GASTRIC HISTAMINE.
42. Carl Bredo Dahl: ANIMAL MODELS IN PSYCHIATRY.

1989

43. Torbjørn A. Fredriksen: CERVICOGENIC HEADACHE.
44. Rolf A. Walstad: CEFTAZIDIME.
45. Rolf Salvesen: THE PUPIL IN CLUSTER HEADACHE.
46. Nils Petter Jørgensen: DRUG EXPOSURE IN EARLY PREGNANCY.
47. Johan C. Ræder: PREMEDICATION AND GENERAL ANAESTHESIA IN OUTPATIENT GYNECOLOGICAL SURGERY.
48. M. R. Shalaby: IMMUNOREGULATORY PROPERTIES OF TNF-α AND THE RELATED CYTOKINES.
49. Anders Waage: THE COMPLEX PATTERN OF CYTOKINES IN SEPTIC SHOCK.
50. Bjarne Christian Eriksen: ELECTROSTIMULATION OF THE PELVIC FLOOR IN FEMALE URINARY INCONTINENCE.
51. Tore B. Halvorsen: PROGNOSTIC FACTORS IN COLORECTAL CANCER.

1990

52. Asbjørn Nordby: CELLULAR TOXICITY OF ROENTGEN CONTRAST MEDIA.
53. Kåre E. Tvedt: X-RAY MICROANALYSIS OF BIOLOGICAL MATERIAL.
54. Tore C. Stiles: COGNITIVE VULNERABILITY FACTORS IN THE DEVELOPMENT AND MAINTENANCE OF DEPRESSION.
55. Eva Hofsli: TUMOR NECROSIS FACTOR AND MULTIDRUG RESISTANCE.
56. Helge S. Haarstad: TROPHIC EFFECTS OF CHOLECYSTOKININ AND SECRETIN ON THE RAT PANCREAS.
57. Lars Engebretsen: TREATMENT OF ACUTE ANTERIOR CRUCIATE LIGAMENT INJURIES.
58. Tarjei Rygnestad: DELIBERATE SELF-POISONING IN TRONDHEIM.
59. Arne Z. Henriksen: STUDIES ON CONSERVED ANTIGENIC DOMAINS ON MAJOR OUTER MEMBRANE PROTEINS FROM ENTEROBACTERIA.
60. Steinar Westin: UNEMPLOYMENT AND HEALTH: Medical and social consequences of a factory closure in a ten-year controlled follow-up study.
61. Ylva Sahlin: INJURY REGISTRATION, a tool for accident preventive work.
62. Helge Bjørnstad Pettersen: BIOSYNTHESIS OF COMPLEMENT BY HUMAN ALVEOLAR MACROPHAGES WITH SPECIAL REFERENCE TO SARCOIDOSIS.
63. Berit Schei: TRAPPED IN PAINFUL LOVE.
64. Lars J. Vatten: PROSPECTIVE STUDIES OF THE RISK OF BREAST CANCER IN A COHORT OF NORWEGIAN WOMAN.

1991

65. Kåre Bergh: APPLICATIONS OF ANTI-C5a SPECIFIC MONOCLONAL ANTIBODIES FOR THE ASSESSMENT OF COMPLEMENT ACTIVATION.
66. Svein Svenningsen: THE CLINICAL SIGNIFICANCE OF INCREASED FEMORAL ANTEVERSION.
67. Olbjørn Klepp: NONSEMINOMATOUS GERM CELL TESTIS CANCER: THERAPEUTIC OUTCOME AND PROGNOSTIC FACTORS.
68. Trond Sand: THE EFFECTS OF CLICK POLARITY ON BRAINSTEM AUDITORY EVOKED POTENTIALS AMPLITUDE, DISPERSION, AND LATENCY VARIABLES.
69. Kjetil B. Åsbakk: STUDIES OF A PROTEIN FROM PSORIATIC SCALE, PSO P27, WITH RESPECT TO ITS POTENTIAL ROLE IN IMMUNE REACTIONS IN PSORIASIS.
70. Arnulf Hestnes: STUDIES ON DOWN´S SYNDROME.
71. Randi Nygaard: LONG-TERM SURVIVAL IN CHILDHOOD LEUKEMIA.
72. Bjørn Hagen: THIO-TEPA.
73. Svein Anda: EVALUATION OF THE HIP JOINT BY COMPUTED TOMOGRAMPHY AND ULTRASONOGRAPHY.

1992

74. Martin Svartberg: AN INVESTIGATION OF PROCESS AND OUTCOME OF SHORT-TERM PSYCHODYNAMIC PSYCHOTHERAPY.
75. Stig Arild Slørdahl: AORTIC REGURGITATION.
76. Harold C Sexton: STUDIES RELATING TO THE TREATMENT OF SYMPTOMATIC NON-PSYCHOTIC PATIENTS.
77. Maurice B. Vincent: VASOACTIVE PEPTIDES IN THE OCULAR/FOREHEAD AREA.
78. Terje Johannessen: CONTROLLED TRIALS IN SINGLE SUBJECTS.
79. Turid Nilsen: PYROPHOSPHATE IN HEPATOCYTE IRON METABOLISM.
80. Olav Haraldseth: NMR SPECTROSCOPY OF CEREBRAL ISCHEMIA AND REPERFUSION IN RAT.
81. Eiliv Brenna: REGULATION OF FUNCTION AND GROWTH OF THE OXYNTIC MUCOSA.

1993

82. Gunnar Bovim: CERVICOGENIC HEADACHE.
83. Jarl Arne Kahn: ASSISTED PROCREATION.
84. Bjørn Naume: IMMUNOREGULATORY EFFECTS OF CYTOKINES ON NK CELLS.
85. Rune Wiseth: AORTIC VALVE REPLACEMENT.
86. Jie Ming Shen: BLOOD FLOW VELOCITY AND RESPIRATORY STUDIES.
87. Piotr Kruszewski: SUNCT SYNDROME WITH SPECIAL REFERENCE TO THE AUTONOMIC NERVOUS SYSTEM.
88. Mette Haase Moen: ENDOMETRIOSIS.
89. Anne Vik: VASCULAR GAS EMBOLISM DURING AIR INFUSION AND AFTER DECOMPRESSION IN PIGS.
90. Lars Jacob Stovner: THE CHIARI TYPE I MALFORMATION.
91. Kjell Å. Salvesen: ROUTINE ULTRASONOGRAPHY IN UTERO AND DEVELOPMENT IN CHILDHOOD.

1994

92. Nina-Beate Liabakk: DEVELOPMENT OF IMMUNOASSAYS FOR TNF AND ITS SOLUBLE RECEPTORS.
93. Sverre Helge Torp: *erb*B ONCOGENES IN HUMAN GLIOMAS AND MENINGIOMAS.
94. Olav M. Linaker: MENTAL RETARDATION AND PSYCHIATRY. Past and present.
95. Per Oscar Feet: INCREASED ANTIDEPRESSANT AND ANTIPANIC EFFECT IN COMBINED TREATMENT WITH DIXYRAZINE AND TRICYCLIC ANTIDEPRESSANTS.
96. Stein Olav Samstad: CROSS SECTIONAL FLOW VELOCITY PROFILES FROM TWO-DIMENSIONAL DOPPLER ULTRASOUND: Studies on early mitral blood flow.
97. Bjørn Backe: STUDIES IN ANTENATAL CARE.
98. Gerd Inger Ringdal: QUALITY OF LIFE IN CANCER PATIENTS.
99. Torvid Kiserud: THE DUCTUS VENOSUS IN THE HUMAN FETUS.
100. Hans E. Fjøsne: HORMONAL REGULATION OF PROSTATIC METABOLISM.
101. Eylert Brodtkorb: CLINICAL ASPECTS OF EPILEPSY IN THE MENTALLY RETARDED.
102. Roar Juul: PEPTIDERGIC MECHANISMS IN HUMAN SUBARACHNOID HEMORRHAGE.
103. Unni Syversen: CHROMOGRANIN A. Phsysiological and Clinical Role.

1995

104. Odd Gunnar Brakstad: THERMOSTABLE NUCLEASE AND THE *nuc* GENE IN THE DIAGNOSIS OF *Staphylococcus aureus* INFECTIONS.
105. Terje Engan: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF PLASMA IN MALIGNANT DISEASE.
106. Kirsten Rasmussen: VIOLENCE IN THE MENTALLY DISORDERED.
107. Finn Egil Skjeldestad: INDUCED ABORTION: Timetrends and Determinants.
108. Roar Stenseth: THORACIC EPIDURAL ANALGESIA IN AORTOCORONARY BYPASS SURGERY.
109. Arild Faxvaag: STUDIES OF IMMUNE CELL FUNCTION *in mice infected with* MURINE RETROVIRUS.

1996

110. Svend Aakhus: NONINVASIVE COMPUTERIZED ASSESSMENT OF LEFT VENTRICULAR FUNCTION AND SYSTEMIC ARTERIAL PROPERTIES. Methodology and some clinical applications.
111. Klaus-Dieter Bolz: INTRAVASCULAR ULTRASONOGRAPHY.
112. Petter Aadahl: CARDIOVASCULAR EFFECTS OF THORACIC AORTIC CROSS-CLAMPING.
113. Sigurd Steinshamn: CYTOKINE MEDIATORS DURING GRANULOCYTOPENIC INFECTIONS.
114. Hans Stifoss-Hanssen: SEEKING MEANING OR HAPPINESS?
115. Anne Kvikstad: LIFE CHANGE EVENTS AND MARITAL STATUS IN RELATION TO RISK AND PROGNOSIS OF CANCER.
116. Torbjørn Grøntvedt: TREATMENT OF ACUTE AND CHRONIC ANTERIOR CRUCIATE LIGAMENT INJURIES. A clinical and biomechanical study.
117. Sigrid Hørven Wigers: CLINICAL STUDIES OF FIBROMYALGIA WITH FOCUS ON ETIOLOGY, TREATMENT AND OUTCOME.
118. Jan Schjøtt: MYOCARDIAL PROTECTION: Functional and Metabolic Characteristics of Two Endogenous Protective Principles.
119. Marit Martinussen: STUDIES OF INTESTINAL BLOOD FLOW AND ITS RELATION TO TRANSITIONAL CIRCULATORY ADAPATION IN NEWBORN INFANTS.
120. Tomm B. Müller: MAGNETIC RESONANCE IMAGING IN FOCAL CEREBRAL ISCHEMIA.
121. Rune Haaverstad: OEDEMA FORMATION OF THE LOWER EXTREMITIES.
122. Magne Børset: THE ROLE OF CYTOKINES IN MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO HEPATOCYTE GROWTH FACTOR.
123. Geir Smedslund: A THEORETICAL AND EMPIRICAL INVESTIGATION OF SMOKING, STRESS AND DISEASE: RESULTS FROM A POPULATION SURVEY.

1997

124. Torstein Vik: GROWTH, MORBIDITY, AND PSYCHOMOTOR DEVELOPMENT IN INFANTS WHO WERE GROWTH RETARDED *IN UTERO*.
125. Siri Forsmo: ASPECTS AND CONSEQUENCES OF OPPORTUNISTIC SCREENING FOR CERVICAL CANCER. Results based on data from three Norwegian counties.
126. Jon S. Skranes: CEREBRAL MRI AND NEURODEVELOPMENTAL OUTCOME IN VERY LOW BIRTH WEIGHT (VLBW) CHILDREN. A follow-up study of a geographically based year cohort of VLBW children at ages one and six years.
127. Knut Bjørnstad: COMPUTERIZED ECHOCARDIOGRAPHY FOR EVALUTION OF CORONARY ARTERY DISEASE.
128. Grethe Elisabeth Borchgrevink: DIAGNOSIS AND TREATMENT OF WHIPLASH/NECK SPRAIN INJURIES CAUSED BY CAR ACCIDENTS.
129. Tor Elsås: NEUROPEPTIDES AND NITRIC OXIDE SYNTHASE IN OCULAR AUTONOMIC AND SENSORY NERVES.
130. Rolf W. Gråwe: EPIDEMIOLOGICAL AND NEUROPSYCHOLOGICAL PERSPECTIVES ON SCHIZOPHRENIA.
131. Tonje Strømholm: CEREBRAL HAEMODYNAMICS DURING THORACIC AORTIC CROSSCLAMPING. An experimental study in pigs

1998

132. Martinus Bråten: STUDIES ON SOME PROBLEMS REALTED TO INTRAMEDULLARY NAILING OF FEMORAL FRACTURES.

133. Ståle Nordgård: PROLIFERATIVE ACTIVITY AND DNA CONTENT AS PROGNOSTIC INDICATORS IN ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK.
134. Egil Lien: SOLUBLE RECEPTORS FOR **TNF** AND **LPS**: RELEASE PATTERN AND POSSIBLE SIGNIFICANCE IN DISEASE.
135. Marit Bjørgaas: HYPOGLYCAEMIA IN CHILDREN WITH DIABETES MELLITUS
136. Frank Skorpen: GENETIC AND FUNCTIONAL ANALYSES OF DNA REPAIR IN HUMAN CELLS.
137. Juan A. Pareja: SUNCT SYNDROME. ON THE CLINICAL PICTURE. ITS DISTINCTION FROM OTHER, SIMILAR HEADACHES.
138. Anders Angelsen: NEUROENDOCRINE CELLS IN HUMAN PROSTATIC CARCINOMAS AND THE PROSTATIC COMPLEX OF RAT, GUINEA PIG, CAT AND DOG.
139. Fabio Antonaci: CHRONIC PAROXYSMAL HEMICRANIA AND HEMICRANIA CONTINUA: TWO DIFFERENT ENTITIES?
140. Sven M. Carlsen: ENDOCRINE AND METABOLIC EFFECTS OF METFORMIN WITH SPECIAL EMPHASIS ON CARDIOVASCULAR RISK FACTORES.

1999

141. Terje A. Murberg: DEPRESSIVE SYMPTOMS AND COPING AMONG PATIENTS WITH CONGESTIVE HEART FAILURE.
142. Harm-Gerd Karl Blaas: THE EMBRYONIC EXAMINATION. Ultrasound studies on the development of the human embryo.
143. Noèmi Becser Andersen:THE CEPHALIC SENSORY NERVES IN UNILATERAL HEADACHES. Anatomical background and neurophysiological evaluation.
144. Eli-Janne Fiskerstrand: LASER TREATMENT OF PORT WINE STAINS. A study of the efficacy and limitations of the pulsed dye laser. Clinical and morfological analyses aimed at improving the therapeutic outcome.
145. Bård Kulseng: A STUDY OF ALGINATE CAPSULE PROPERTIES AND CYTOKINES IN RELATION TO INSULIN DEPENDENT DIABETES MELLITUS.
146. Terje Haug: STRUCTURE AND REGULATION OF THE HUMAN UNG GENE ENCODING URACIL-DNA GLYCOSYLASE.
147. Heidi Brurok: MANGANESE AND THE HEART. A Magic Metal with Diagnostic and Therapeutic Possibilites.
148. Agnes Kathrine Lie: DIAGNOSIS AND PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL INTRAEPITELIAL NEOPLASIA. Relationship to Cell Cycle Regulatory Proteins and HLA DQBI Genes.
149. Ronald Mårvik: PHARMACOLOGICAL, PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STUDIES ON ISOLATED STOMACS.
150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

2000

158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING CLINICAL AND EXPERIMENTAL STUDIES
159. xxxxxxxxx (blind number)
160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.
161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.

162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.
168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

2001

178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT

192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM
198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

2002

201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

2003

216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.

217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES
223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

2004

235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA

244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

2005

248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS
251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
262. Ole Johan Kemi: ON THE CELLULAR BASIS OF AEROBIC FITNESS, INTENSITY-DEPENDENCE AND TIME-COURSE OF CARDIOMYOCYTE AND ENDOTHELIAL ADAPTATIONS TO EXERCISE TRAINING
263. Eszter Vanky: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN PREGNANCY
264. Hild Fjærtoft: EXTENDED STROKE UNIT SERVICE AND EARLY SUPPORTED DISCHARGE. SHORT AND LONG-TERM EFFECTS
265. Grete Dyb: POSTTRAUMATIC STRESS REACTIONS IN CHILDREN AND ADOLESCENTS
266. Vidar Fykse: SOMATOSTATIN AND THE STOMACH
267. Kirsti Berg: OXIDATIVE STRESS AND THE ISCHEMIC HEART: A STUDY IN PATIENTS UNDERGOING CORONARY REVASCULARIZATION
268. Björn Inge Gustafsson: THE SEROTONIN PRODUCING ENTEROCHROMAFFIN CELL, AND EFFECTS OF HYPERSEROTONINEMIA ON HEART AND BONE

2006

269. Torstein Baade Rø: EFFECTS OF BONE MORPHOGENETIC PROTEINS, HEPATOCYTE GROWTH FACTOR AND INTERLEUKIN-21 IN MULTIPLE MYELOMA
270. May-Britt Tessem: METABOLIC EFFECTS OF ULTRAVIOLET RADIATION ON THE ANTERIOR PART OF THE EYE
271. Anne-Sofie Helvik: COPING AND EVERYDAY LIFE IN A POPULATION OF ADULTS WITH HEARING IMPAIRMENT

272. Therese Standal: MULTIPLE MYELOMA: THE INTERPLAY BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MARROW MICROENVIRONMENT
273. Ingvild Saltvedt: TREATMENT OF ACUTELY SICK, FRAIL ELDERLY PATIENTS IN A GERIATRIC EVALUATION AND MANAGEMENT UNIT – RESULTS FROM A PROSPECTIVE RANDOMISED TRIAL
274. Birger Henning Endreseth: STRATEGIES IN RECTAL CANCER TREATMENT – FOCUS ON EARLY RECTAL CANCER AND THE INFLUENCE OF AGE ON PROGNOSIS
275. Anne Mari Aukan Rokstad: ALGINATE CAPSULES AS BIOREACTORS FOR CELL THERAPY
276. Mansour Akbari: HUMAN BASE EXCISION REPAIR FOR PRESERVATION OF GENOMIC STABILITY
277. Stein Sundstrøm: IMPROVING TREATMENT IN PATIENTS WITH LUNG CANCER – RESULTS FROM TWO MULITCENTRE RANDOMISED STUDIES
278. Hilde Pleym: BLEEDING AFTER CORONARY ARTERY BYPASS SURGERY - STUDIES ON HEMOSTATIC MECHANISMS, PROPHYLACTIC DRUG TREATMENT AND EFFECTS OF AUTOTRANSFUSION
279. Line Merethe Oldervoll: PHYSICAL ACTIVITY AND EXERCISE INTERVENTIONS IN CANCER PATIENTS
280. Boye Welde: THE SIGNIFICANCE OF ENDURANCE TRAINING, RESISTANCE TRAINING AND MOTIVATIONAL STYLES IN ATHLETIC PERFORMANCE AMONG ELITE JUNIOR CROSS-COUNTRY SKIERS
281. Per Olav Vandvik: IRRITABLE BOWEL SYNDROME IN NORWAY, STUDIES OF PREVALENCE, DIAGNOSIS AND CHARACTERISTICS IN GENERAL PRACTICE AND IN THE POPULATION
282. Idar Kirkeby-Garstad: CLINICAL PHYSIOLOGY OF EARLY MOBILIZATION AFTER CARDIAC SURGERY
283. Linn Getz: SUSTAINABLE AND RESPONSIBLE PREVENTIVE MEDICINE. CONCEPTUALISING ETHICAL DILEMMAS ARISING FROM CLINICAL IMPLEMENTATION OF ADVANCING MEDICAL TECHNOLOGY
284. Eva Tegnander: DETECTION OF CONGENITAL HEART DEFECTS IN A NON-SELECTED POPULATION OF 42,381 FETUSES
285. Kristin Gabestad Nørsett: GENE EXPRESSION STUDIES IN GASTROINTESTINAL PATHOPHYSIOLOGY AND NEOPLASIA
286. Per Magnus Haram: GENETIC VS. AQUIRED FITNESS: METABOLIC, VASCULAR AND CARDIOMYOCYTE ADAPTATIONS
287. Agneta Johansson: GENERAL RISK FACTORS FOR GAMBLING PROBLEMS AND THE PREVALENCE OF PATHOLOGICAL GAMBLING IN NORWAY
288. Svein Artur Jensen: THE PREVALENCE OF SYMPTOMATIC ARTERIAL DISEASE OF THE LOWER LIMB
289. Charlotte Björk Ingul: QUANITIFICATION OF REGIONAL MYOCARDIAL FUNCTION BY STRAIN RATE AND STRAIN FOR EVALUATION OF CORONARY ARTERY DISEASE. AUTOMATED VERSUS MANUAL ANALYSIS DURING ACUTE MYOCARDIAL INFARCTION AND DOBUTAMINE STRESS ECHOCARDIOGRAPHY
290. Jakob Nakling: RESULTS AND CONSEQUENCES OF ROUTINE ULTRASOUND SCREENING IN PREGNANCY – A GEOGRAPHIC BASED POPULATION STUDY
291. Anne Engum: DEPRESSION AND ANXIETY – THEIR RELATIONS TO THYROID DYSFUNCTION AND DIABETES IN A LARGE EPIDEMIOLOGICAL STUDY
292. Ottar Bjerkeset: ANXIETY AND DEPRESSION IN THE GENERAL POPULATION: RISK FACTORS, INTERVENTION AND OUTCOME – THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
293. Jon Olav Drogset: RESULTS AFTER SURGICAL TREATMENT OF ANTERIOR CRUCIATE LIGAMENT INJURIES – A CLINICAL STUDY
294. Lars Fosse: MECHANICAL BEHAVIOUR OF COMPACTED MORSELLISED BONE – AN EXPERIMENTAL IN VITRO STUDY
295. Gunilla Klensmeden Fosse: MENTAL HEALTH OF PSYCHIATRIC OUTPATIENTS BULLIED IN CHILDHOOD
296. Paul Jarle Mork: MUSCLE ACTIVITY IN WORK AND LEISURE AND ITS ASSOCIATION TO MUSCULOSKELETAL PAIN

297. Björn Stenström: LESSONS FROM RODENTS: I: MECHANISMS OF OBESITY SURGERY – ROLE OF STOMACH. II: CARCINOGENIC EFFECTS OF *HELICOBACTER PYLORI* AND SNUS IN THE STOMACH

2007

298. Haakon R. Skogseth: INVASIVE PROPERTIES OF CANCER – A TREATMENT TARGET ? IN VITRO STUDIES IN HUMAN PROSTATE CANCER CELL LINES
299. Janniche Hammer: GLUTAMATE METABOLISM AND CYCLING IN MESIAL TEMPORAL LOBE EPILEPSY
300. May Britt Drugli: YOUNG CHILDREN TREATED BECAUSE OF ODD/CD: CONDUCT PROBLEMS AND SOCIAL COMPETENCIES IN DAY-CARE AND SCHOOL SETTINGS
301. Arne Skjold: MAGNETIC RESONANCE KINETICS OF MANGANESE DIPYRIDOXYL DIPHOSPHATE (MnDPDP) IN HUMAN MYOCARDIUM. STUDIES IN HEALTHY VOLUNTEERS AND IN PATIENTS WITH RECENT MYOCARDIAL INFARCTION
302. Siri Malm: LEFT VENTRICULAR SYSTOLIC FUNCTION AND MYOCARDIAL PERFUSION ASSESSED BY CONTRAST ECHOCARDIOGRAPHY
303. Valentina Maria do Rosario Cabral Iversen: MENTAL HEALTH AND PSYCHOLOGICAL ADAPTATION OF CLINICAL AND NON-CLINICAL MIGRANT GROUPS
304. Lasse Løvstakken: SIGNAL PROCESSING IN DIAGNOSTIC ULTRASOUND: ALGORITHMS FOR REAL-TIME ESTIMATION AND VISUALIZATION OF BLOOD FLOW VELOCITY
305. Elisabeth Olstad: GLUTAMATE AND GABA: MAJOR PLAYERS IN NEURONAL METABOLISM
306. Lilian Leistad: THE ROLE OF CYTOKINES AND PHOSPHOLIPASE A_2S IN ARTICULAR CARTILAGE CHONDROCYTES IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS
307. Arne Vaaler: EFFECTS OF PSYCHIATRIC INTENSIVE CARE UNIT IN AN ACUTE PSYCIATHRIC WARD
308. Mathias Toft: GENETIC STUDIES OF LRRK2 AND PINK1 IN PARKINSON'S DISEASE
309. Ingrid Løvold Mostad: IMPACT OF DIETARY FAT QUANTITY AND QUALITY IN TYPE 2 DIABETES WITH EMPHASIS ON MARINE N-3 FATTY ACIDS
310. Torill Eidhammer Sjøbakk: MR DETERMINED BRAIN METABOLIC PATTERN IN PATIENTS WITH BRAIN METASTASES AND ADOLESCENTS WITH LOW BIRTH WEIGHT
311. Vidar Beisvåg: PHYSIOLOGICAL GENOMICS OF HEART FAILURE: FROM TECHNOLOGY TO PHYSIOLOGY
312. Olav Magnus Søndenå Fredheim: HEALTH RELATED QUALITY OF LIFE ASSESSMENT AND ASPECTS OF THE CLINICAL PHARMACOLOGY OF METHADONE IN PATIENTS WITH CHRONIC NON-MALIGNANT PAIN
313. Anne Brantberg: FETAL AND PERINATAL IMPLICATIONS OF ANOMALIES IN THE GASTROINTESTINAL TRACT AND THE ABDOMINAL WALL
314. Erik Solligård: GUT LUMINAL MICRODIALYSIS
315. Elin Tollefsen: RESPIRATORY SYMPTOMS IN A COMPREHENSIVE POPULATION BASED STUDY AMONG ADOLESCENTS 13-19 YEARS. YOUNG-HUNT 1995-97 AND 2000-01; THE NORD-TRØNDELAG HEALTH STUDIES (HUNT)
316. Anne-Tove Brenne: GROWTH REGULATION OF MYELOMA CELLS
317. Heidi Knobel: FATIGUE IN CANCER TREATMENT – ASSESSMENT, COURSE AND ETIOLOGY
318. Torbjørn Dahl: CAROTID ARTERY STENOSIS. DIAGNOSTIC AND THERAPEUTIC ASPECTS
319. Inge-Andre Rasmussen jr.: FUNCTIONAL AND DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING IN NEUROSURGICAL PATIENTS
320. Grete Helen Bratberg: PUBERTAL TIMING – ANTECEDENT TO RISK OR RESILIENCE ? EPIDEMIOLOGICAL STUDIES ON GROWTH, MATURATION AND HEALTH RISK BEHAVIOURS; THE YOUNG HUNT STUDY, NORD-TRØNDELAG, NORWAY
321. Sveinung Sørhaug: THE PULMONARY NEUROENDOCRINE SYSTEM. PHYSIOLOGICAL, PATHOLOGICAL AND TUMOURIGENIC ASPECTS
322. Olav Sande Eftedal: ULTRASONIC DETECTION OF DECOMPRESSION INDUCED VASCULAR MICROBUBBLES
323. Rune Bang Leistad: PAIN, AUTONOMIC ACTIVATION AND MUSCULAR ACTIVITY RELATED TO EXPERIMENTALLY-INDUCED COGNITIVE STRESS IN HEADACHE PATIENTS

324. Svein Brekke: TECHNIQUES FOR ENHANCEMENT OF TEMPORAL RESOLUTION IN THREE-DIMENSIONAL ECHOCARDIOGRAPHY
325. Kristian Bernhard Nilsen: AUTONOMIC ACTIVATION AND MUSCLE ACTIVITY IN RELATION TO MUSCULOSKELETAL PAIN
326. Anne Irene Hagen: HEREDITARY BREAST CANCER IN NORWAY. DETECTION AND PROGNOSIS OF BREAST CANCER IN FAMILIES WITH *BRCA1*GENE MUTATION
327. Ingebjørg S. Juel : INTESTINAL INJURY AND RECOVERY AFTER ISCHEMIA. AN EXPERIMENTAL STUDY ON RESTITUTION OF THE SURFACE EPITHELIUM, INTESTINAL PERMEABILITY, AND RELEASE OF BIOMARKERS FROM THE MUCOSA
328. Runa Heimstad: POST-TERM PREGNANCY
329. Jan Egil Afset: ROLE OF ENTEROPATHOGENIC *ESCHERICHIA COLI* IN CHILDHOOD DIARRHOEA IN NORWAY
330. Bent Håvard Hellum: *IN VITRO* INTERACTIONS BETWEEN MEDICINAL DRUGS AND HERBS ON CYTOCHROME P-450 METABOLISM AND P-GLYCOPROTEIN TRANSPORT
331. Morten André Høydal: CARDIAC DYSFUNCTION AND MAXIMAL OXYGEN UPTAKE MYOCARDIAL ADAPTATION TO ENDURANCE TRAINING

2008

332. Andreas Møllerløkken: REDUCTION OF VASCULAR BUBBLES: METHODS TO PREVENT THE ADVERSE EFFECTS OF DECOMPRESSION
333. Anne Hege Aamodt: COMORBIDITY OF HEADACHE AND MIGRAINE IN THE NORD-TRØNDELAG HEALTH STUDY 1995-97
334. Brage Høyem Amundsen: MYOCARDIAL FUNCTION QUANTIFIED BY SPECKLE TRACKING AND TISSUE DOPPLER ECHOCARDIOGRAPHY – VALIDATION AND APPLICATION IN EXERCISE TESTING AND TRAINING
335. Inger Anne Næss: INCIDENCE, MORTALITY AND RISK FACTORS OF FIRST VENOUS THROMBOSIS IN A GENERAL POPULATION. RESULTS FROM THE SECOND NORD-TRØNDELAG HEALTH STUDY (HUNT2)
336. Vegard Bugten: EFFECTS OF POSTOPERATIVE MEASURES AFTER FUNCTIONAL ENDOSCOPIC SINUS SURGERY
337. Morten Bruvold: MANGANESE AND WATER IN CARDIAC MAGNETIC RESONANCE IMAGING
338. Miroslav Fris: THE EFFECT OF SINGLE AND REPEATED ULTRAVIOLET RADIATION ON THE ANTERIOR SEGMENT OF THE RABBIT EYE
339. Svein Arne Aase: METHODS FOR IMPROVING QUALITY AND EFFICIENCY IN QUANTITATIVE ECHOCARDIOGRAPHY – ASPECTS OF USING HIGH FRAME RATE
340. Roger Almvik: ASSESSING THE RISK OF VIOLENCE: DEVELOPMENT AND VALIDATION OF THE BRØSET VIOLENCE CHECKLIST
341. Ottar Sundheim: STRUCTURE-FUNCTION ANALYSIS OF HUMAN ENZYMES INITIATING NUCLEOBASE REPAIR IN DNA AND RNA
342. Anne Mari Undheim: SHORT AND LONG-TERM OUTCOME OF EMOTIONAL AND BEHAVIOURAL PROBLEMS IN YOUNG ADOLESCENTS WITH AND WITHOUT READING DIFFICULTIES
343. Helge Garåsen: THE TRONDHEIM MODEL. IMPROVING THE PROFESSIONAL COMMUNICATION BETWEEN THE VARIOUS LEVELS OF HEALTH CARE SERVICES AND IMPLEMENTATION OF INTERMEDIATE CARE AT A COMMUNITY HOSPITAL COULD PROVIDE BETTER CARE FOR OLDER PATIENTS. SHORT AND LONG TERM EFFECTS
344. Olav A. Foss: "THE ROTATION RATIOS METHOD". A METHOD TO DESCRIBE ALTERED SPATIAL ORIENTATION IN SEQUENTIAL RADIOGRAPHS FROM ONE PELVIS
345. Bjørn Olav Åsvold: THYROID FUNCTION AND CARDIOVASCULAR HEALTH
346. Torun Margareta Melø: NEURONAL GLIAL INTERACTIONS IN EPILEPSY
347. Irina Poliakova Eide: FETAL GROWTH RESTRICTION AND PRE-ECLAMPSIA: SOME CHARACTERISTICS OF FETO-MATERNAL INTERACTIONS IN DECIDUA BASALIS
348. Torunn Askim: RECOVERY AFTER STROKE. ASSESSMENT AND TREATMENT; WITH FOCUS ON MOTOR FUNCTION
349. Ann Elisabeth Åsberg: NEUTROPHIL ACTIVATION IN A ROLLER PUMP MODEL OF CARDIOPULMONARY BYPASS. INFLUENCE ON BIOMATERIAL, PLATELETS AND COMPLEMENT

350. Lars Hagen: REGULATION OF DNA BASE EXCISION REPAIR BY PROTEIN INTERACTIONS AND POST TRANSLATIONAL MODIFICATIONS
351. Sigrun Beate Kjøtrød: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN ASSISTED REPRODUCTION
352. Steven Keita Nishiyama: PERSPECTIVES ON LIMB-VASCULAR HETEROGENEITY: IMPLICATIONS FOR HUMAN AGING, SEX, AND EXERCISE
353. Sven Peter Näsholm: ULTRASOUND BEAMS FOR ENHANCED IMAGE QUALITY
354. Jon Ståle Ritland: PRIMARY OPEN-ANGLE GLAUCOMA & EXFOLIATIVE GLAUCOMA. SURVIVAL, COMORBIDITY AND GENETICS
355. Sigrid Botne Sando: ALZHEIMER'S DISEASE IN CENTRAL NORWAY. GENETIC AND EDUCATIONAL ASPECTS
356. Parvinder Kaur: CELLULAR AND MOLECULAR MECHANISMS BEHIND METHYLMERCURY-INDUCED NEUROTOXICITY
357. Ismail Cüneyt Güzey: DOPAMINE AND SEROTONIN RECEPTOR AND TRANSPORTER GENE POLYMORPHISMS AND EXTRAPYRAMIDAL SYMPTOMS. STUDIES IN PARKINSON'S DISEASE AND IN PATIENTS TREATED WITH ANTIPSYCHOTIC OR ANTIDEPRESSANT DRUGS
358. Brit Dybdahl: EXTRA-CELLULAR INDUCIBLE HEAT-SHOCK PROTEIN 70 (Hsp70) – A ROLE IN THE INFLAMMATORY RESPONSE ?
359. Kristoffer Haugarvoll: IDENTIFYING GENETIC CAUSES OF PARKINSON'S DISEASE IN NORWAY
360. Nadra Nilsen: TOLL-LIKE RECEPTOR 2 –EXPRESSION, REGULATION AND SIGNALING
361. Johan Håkon Bjørngaard: PATIENT SATISFACTION WITH OUTPATIENT MENTAL HEALTH SERVICES – THE INFLUENCE OF ORGANIZATIONAL FACTORS.
362. Kjetil Høydal : EFFECTS OF HIGH INTENSITY AEROBIC TRAINING IN HEALTHY SUBJECTS AND CORONARY ARTERY DISEASE PATIENTS; THE IMPORTANCE OF INTENSITY,, DURATION AND FREQUENCY OF TRAINING.
363. Trine Karlsen: TRAINING IS MEDICINE: ENDURANCE AND STRENGTH TRAINING IN CORONARY ARTERY DISEASE AND HEALTH.
364. Marte Thuen: MANGANASE-ENHANCED AND DIFFUSION TENSOR MR IMAGING OF THE NORMAL, INJURED AND REGENERATING RAT VISUAL PATHWAY
365. Cathrine Broberg Vågbø: DIRECT REPAIR OF ALKYLATION DAMAGE IN DNA AND RNA BY 2-OXOGLUTARATE- AND IRON-DEPENDENT DIOXYGENASES
366. Arnt Erik Tjønna: AEROBIC EXERCISE AND CARDIOVASCULAR RISK FACTORS IN OVERWEIGHT AND OBESE ADOLESCENTS AND ADULTS
367. Marianne W. Furnes: FEEDING BEHAVIOR AND BODY WEIGHT DEVELOPMENT: LESSONS FROM RATS
368. Lene N. Johannessen: FUNGAL PRODUCTS AND INFLAMMATORY RESPONSES IN HUMAN MONOCYTES AND EPITHELIAL CELLS
369. Anja Bye: GENE EXPRESSION PROFILING OF *INHERITED* AND *ACQUIRED* MAXIMAL OXYGEN UPTAKE – RELATIONS TO THE METABOLIC SYNDROME.
370. Oluf Dimitri Røe: MALIGNANT MESOTHELIOMA: VIRUS, BIOMARKERS AND GENES. A TRANSLATIONAL APPROACH
371. Ane Cecilie Dale: DIABETES MELLITUS AND FATAL ISCHEMIC HEART DISEASE. ANALYSES FROM THE HUNT1 AND 2 STUDIES
372. Jacob Christian Hølen: PAIN ASSESSMENT IN PALLIATIVE CARE: VALIDATION OF METHODS FOR SELF-REPORT AND BEHAVIOURAL ASSESSMENT
373. Erming Tian: THE GENETIC IMPACTS IN THE ONCOGENESIS OF MULTIPLE MYELOMA
374. Ole Bosnes: KLINISK UTPRØVING AV NORSKE VERSJONER AV NOEN SENTRALE TESTER PÅ KOGNITIV FUNKSJON
375. Ola M. Rygh: 3D ULTRASOUND BASED NEURONAVIGATION IN NEUROSURGERY. A CLINICAL EVALUATION
376. Astrid Kamilla Stunes: ADIPOKINES, PEROXISOME PROFILERATOR ACTIVATED RECEPTOR (PPAR) AGONISTS AND SEROTONIN. COMMON REGULATORS OF BONE AND FAT METABOLISM
377. Silje Engdal: HERBAL REMEDIES USED BY NORWEGIAN CANCER PATIENTS AND THEIR ROLE IN HERB-DRUG INTERACTIONS
378. Kristin Offerdal: IMPROVED ULTRASOUND IMAGING OF THE FETUS AND ITS CONSEQUENCES FOR SEVERE AND LESS SEVERE ANOMALIES

379. Øivind Rognmo: HIGH-INTENSITY AEROBIC EXERCISE AND CARDIOVASCULAR HEALTH
380. Jo-Åsmund Lund: RADIOTHERAPY IN ANAL CARCINOMA AND PROSTATE CANCER

2009

381. Tore Grüner Bjåstad: HIGH FRAME RATE ULTRASOUND IMAGING USING PARALLEL BEAMFORMING
382. Erik Søndenaa: INTELLECTUAL DISABILITIES IN THE CRIMINAL JUSTICE SYSTEM
383. Berit Rostad: SOCIAL INEQUALITIES IN WOMEN'S HEALTH, HUNT 1984-86 AND 1995-97, THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
384. Jonas Crosby: ULTRASOUND-BASED QUANTIFICATION OF MYOCARDIAL DEFORMATION AND ROTATION
385. Erling Tronvik: MIGRAINE, BLOOD PRESSURE AND THE RENIN-ANGIOTENSIN SYSTEM
386. Tom Christensen: BRINGING THE GP TO THE FOREFRONT OF EPR DEVELOPMENT
387. Håkon Bergseng: ASPECTS OF GROUP B STREPTOCOCCUS (GBS) DISEASE IN THE NEWBORN. EPIDEMIOLOGY, CHARACTERISATION OF INVASIVE STRAINS AND EVALUATION OF INTRAPARTUM SCREENING
388. Ronny Myhre: GENETIC STUDIES OF CANDIDATE TENE3S IN PARKINSON'S DISEASE
389. Torbjørn Moe Eggebø: ULTRASOUND AND LABOUR
390. Eivind Wang: TRAINING IS MEDICINE FOR PATIENTS WITH PERIPHERAL ARTERIAL DISEASE
391. Thea Kristin Våtsveen: GENETIC ABERRATIONS IN MYELOMA CELLS
392. Thomas Jozefiak: QUALITY OF LIFE AND MENTAL HEALTH IN CHILDREN AND ADOLESCENTS: CHILD AND PARENT PERSPECTIVES
393. Jens Erik Slagsvold: N-3 POLYUNSATURATED FATTY ACIDS IN HEALTH AND DISEASE – CLINICAL AND MOLECULAR ASPECTS
394. Kristine Misund: A STUDY OF THE TRANSCRIPTIONAL REPRESSOR ICER. REGULATORY NETWORKS IN GASTRIN-INDUCED GENE EXPRESSION
395. Franco M. Impellizzeri: HIGH-INTENSITY TRAINING IN FOOTBALL PLAYERS. EFFECTS ON PHYSICAL AND TECHNICAL PERFORMANCE
396. Kari Hanne Gjeilo: HEALTH-RELATED QUALITY OF LIFE AND CHRONIC PAIN IN PATIENTS UNDERGOING CARDIAC SURGERY
397. Øyvind Hauso: NEUROENDOCRINE ASPECTS OF PHYSIOLOGY AND DISEASE
398. Ingvild Bjellmo Johnsen: INTRACELLULAR SIGNALING MECHANISMS IN THE INNATE IMMUNE RESPONSE TO VIRAL INFECTIONS
399. Linda Tømmerdal Roten: GENETIC PREDISPOSITION FOR DEVELOPMENT OF PREEMCLAMPSIA – CANDIDATE GENE STUDIES IN THE HUNT (NORD-TRØNDELAG HEALTH STUDY) POPULATION
400. Trude Teoline Nausthaug Rakvåg: PHARMACOGENETICS OF MORPHINE IN CANCER PAIN
401. Hanne Lehn: MEMORY FUNCTIONS OF THE HUMAN MEDIAL TEMPORAL LOBE STUDIED WITH fMRI
402. Randi Utne Holt: ADHESION AND MIGRATION OF MYELOMA CELLS – IN VITRO STUDIES –
403. Trygve Solstad: NEURAL REPRESENTATIONS OF EUCLIDEAN SPACE
404. Unn-Merete Fagerli: MULTIPLE MYELOMA CELLS AND CYTOKINES FROM THE BONE MARROW ENVIRONMENT; ASPECTS OF GROWTH REGULATION AND MIGRATION
405. Sigrid Bjørnelv: EATING– AND WEIGHT PROBLEMS IN ADOLESCENTS, THE YOUNG HUNT-STUDY
406. Mari Hoff: CORTICAL HAND BONE LOSS IN RHEUMATOID ARTHRITIS. EVALUATING DIGITAL X-RAY RADIOGRAMMETRY AS OUTCOME MEASURE OF DISEASE ACTIVITY, RESPONSE VARIABLE TO TREATMENT AND PREDICTOR OF BONE DAMAGE
407. Siri Bjørgen: AEROBIC HIGH INTENSITY INTERVAL TRAINING IS AN EFFECTIVE TREATMENT FOR PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE
408. Susanne Lindqvist: VISION AND BRAIN IN ADOLESCENTS WITH LOW BIRTH WEIGHT
409. Torbjørn Hergum: 3D ULTRASOUND FOR QUANTITATIVE ECHOCARDIOGRAPHY

410. Jørgen Urnes: PATIENT EDUCATION IN GASTRO-OESOPHAGEAL REFLUX DISEASE. VALIDATION OF A DIGESTIVE SYMPTOMS AND IMPACT QUESTIONNAIRE AND A RANDOMISED CONTROLLED TRIAL OF PATIENT EDUCATION
411. Elvar Eyjolfsson: 13C NMRS OF ANIMAL MODELS OF SCHIZOPHRENIA
412. Marius Steiro Fimland: CHRONIC AND ACUTE NEURAL ADAPTATIONS TO STRENGTH TRAINING
413. Øyvind Støren: RUNNING AND CYCLING ECONOMY IN ATHLETES; DETERMINING FACTORS, TRAINING INTERVENTIONS AND TESTING
414. Håkon Hov: HEPATOCYTE GROWTH FACTOR AND ITS RECEPTOR C-MET. AUTOCRINE GROWTH AND SIGNALING IN MULTIPLE MYELOMA CELLS
415. Maria Radtke: ROLE OF AUTOIMMUNITY AND OVERSTIMULATION FOR BETA-CELL DEFICIENCY. EPIDEMIOLOGICAL AND THERAPEUTIC PERSPECTIVES
416. Liv Bente Romundstad: ASSISTED FERTILIZATION IN NORWAY: SAFETY OF THE REPRODUCTIVE TECHNOLOGY
417. Erik Magnus Berntsen: PREOPERATIV PLANNING AND FUNCTIONAL NEURONAVIGATION – WITH FUNCTIONAL MRI AND DIFFUSION TENSOR TRACTOGRAPHY IN PATIENTS WITH BRAIN LESIONS
418. Tonje Strømmen Steigedal: MOLECULAR MECHANISMS OF THE PROLIFERATIVE RESPONSE TO THE HORMONE GASTRIN
419. Vidar Rao: EXTRACORPOREAL PHOTOCHEMOTHERAPY IN PATIENTS WITH CUTANEOUS T CELL LYMPHOMA OR GRAFT-vs-HOST DISEASE
420. Torkild Visnes: DNA EXCISION REPAIR OF URACIL AND 5-FLUOROURACIL IN HUMAN CANCER CELL LINES

2010

421. John Munkhaugen: BLOOD PRESSURE, BODY WEIGHT, AND KIDNEY FUNCTION IN THE NEAR-NORMAL RANGE: NORMALITY, RISK FACTOR OR MORBIDITY ?
422. Ingrid Castberg: PHARMACOKINETICS, DRUG INTERACTIONS AND ADHERENCE TO TREATMENT WITH ANTIPSYCHOTICS: STUDIES IN A NATURALISTIC SETTING
423. Jian Xu: BLOOD-OXYGEN-LEVEL-DEPENDENT-FUNCTIONAL MAGNETIC RESONANCE IMAGING AND DIFFUSION TENSOR IMAGING IN TRAUMATIC BRAIN INJURY RESEARCH
424. Sigmund Simonsen: ACCEPTABLE RISK AND THE REQUIREMENT OF PROPORTIONALITY IN EUROPEAN BIOMEDICAL RESEARCH LAW. WHAT DOES THE REQUIREMENT THAT BIOMEDICAL RESEARCH SHALL NOT INVOLVE RISKS AND BURDENS DISPROPORTIONATE TO ITS POTENTIAL BENEFITS MEAN?
425. Astrid Woodhouse: MOTOR CONTROL IN WHIPLASH AND CHRONIC NON-TRAUMATIC NECK PAIN
426. Line Rørstad Jensen: EVALUATION OF TREATMENT EFFECTS IN CANCER BY MR IMAGING AND SPECTROSCOPY
427. Trine Moholdt: AEROBIC EXERCISE IN CORONARY HEART DISEASE
428. Øystein Olsen: ANALYSIS OF MANGANESE ENHANCED MRI OF THE NORMAL AND INJURED RAT CENTRAL NERVOUS SYSTEM
429. Bjørn H. Grønberg: PEMETREXED IN THE TREATMENT OF ADVANCED LUNG CANCER
430. Vigdis Schnell Husby: REHABILITATION OF PATIENTS UNDERGOING TOTAL HIP ARTHROPLASTY WITH FOCUS ON MUSCLE STRENGTH, WALKING AND AEROBIC ENDURANCE PERFORMANCE
431. Torbjørn Øien: CHALLENGES IN PRIMARY PREVENTION OF ALLERGY. THE PREVENTION OF ALLERGY AMONG CHILDREN IN TRONDHEIM (PACT) STUDY.
432. Kari Anne Indredavik Evensen: BORN TOO SOON OR TOO SMALL: MOTOR PROBLEMS IN ADOLESCENCE
433. Lars Adde: PREDICTION OF CEREBRAL PALSY IN YOUNG INFANTS. COMPUTER BASED ASSESSMENT OF GENERAL MOVEMENTS
434. Magnus Fasting: PRE- AND POSTNATAL RISK FACTORS FOR CHILDHOOD ADIPOSITY
435. Vivi Talstad Monsen: MECHANISMS OF ALKYLATION DAMAGE REPAIR BY HUMAN AlkB HOMOLOGUES
436. Toril Skandsen: MODERATE AND SEVERE TRAUMATIC BRAIN INJURY. MAGNETIC RESONANCE IMAGING FINDINGS, COGNITION AND RISK FACTORS FOR DISABILITY

437. Ingeborg Smidesang: ALLERGY RELATED DISORDERS AMONG 2-YEAR OLDS AND ADOLESCENTS IN MID-NORWAY – PREVALENCE, SEVERITY AND IMPACT. THE PACT STUDY 2005, THE YOUNG HUNT STUDY 1995-97
438. Vidar Halsteinli: MEASURING EFFICIENCY IN MENTAL HEALTH SERVICE DELIVERY: A STUDY OF OUTPATIENT UNITS IN NORWAY
439. Karen Lehrmann Ægidius: THE PREVALENCE OF HEADACHE AND MIGRAINE IN RELATION TO SEX HORMONE STATUS IN WOMEN. THE HUNT 2 STUDY
440. Madelene Ericsson: EXERCISE TRAINING IN GENETIC MODELS OF HEART FAILURE
441. Marianne Klokk: THE ASSOCIATION BETWEEN SELF-REPORTED ECZEMA AND COMMON MENTAL DISORDERS IN THE GENERAL POPULATION. THE HORDALAND HEALTH STUDY (HUSK)
442. Tomas Ottemo Stølen: IMPAIRED CALCIUM HANDLING IN ANIMAL AND HUMAN CARDIOMYOCYTES REDUCE CONTRACTILITY AND INCREASE ARRHYTHMIA POTENTIAL – EFFECTS OF AEROBIC EXERCISE TRAINING
443. Bjarne Hansen: ENHANCING TREATMENT OUTCOME IN COGNITIVE BEHAVIOURAL THERAPY FOR OBSESSIVE COMPULSIVE DISORDER: THE IMPORTANCE OF COGNITIVE FACTORS
444. Mona Løvlien: WHEN EVERY MINUTE COUNTS. FROM SYMPTOMS TO ADMISSION FOR ACUTE MYOCARDIAL INFARCTION WITH SPECIAL EMPHASIS ON GENDER DIFFERECES
445. Karin Margaretha Gilljam: DNA REPAIR PROTEIN COMPLEXES, FUNCTIONALITY AND SIGNIFICANCE FOR REPAIR EFFICIENCY AND CELL SURVIVAL
446. Anne Byriel Walls: NEURONAL GLIAL INTERACTIONS IN CEREBRAL ENERGY – AND AMINO ACID HOMEOSTASIS – IMPLICATIONS OF GLUTAMATE AND GABA
447. Cathrine Fallang Knetter: MECHANISMS OF TOLL-LIKE RECEPTOR 9 ACTIVATION
448. Marit Følsvik Svindseth: A STUDY OF HUMILIATION, NARCISSISM AND TREATMENT OUTCOME IN PATIENTS ADMITTED TO PSYCHIATRIC EMERGENCY UNITS
449. Karin Elvenes Bakkelund: GASTRIC NEUROENDOCRINE CELLS – ROLE IN GASTRIC NEOPLASIA IN MAN AND RODENTS
450. Kirsten Brun Kjelstrup: DORSOVENTRAL DIFFERENCES IN THE SPATIAL REPRESENTATION AREAS OF THE RAT BRAIN
451. Roar Johansen: MR EVALUATION OF BREAST CANCER PATIENTS WITH POOR PROGNOSIS
452. Rigmor Myran: POST TRAUMATIC NECK PAIN. EPIDEMIOLOGICAL, NEURORADIOLOGICAL AND CLINICAL ASPECTS
453. Krisztina Kunszt Johansen: GENEALOGICAL, CLINICAL AND BIOCHEMICAL STUDIES IN *LRRK2* – ASSOCIATED PARKINSON'S DISEASE
454. Pål Gjerden: THE USE OF ANTICHOLINERGIC ANTIPARKINSON AGENTS IN NORWAY. EPIDEMIOLOGY, TOXICOLOGY AND CLINICAL IMPLICATIONS
455. Else Marie Huuse: ASSESSMENT OF TUMOR MICROENVIRONMENT AND TREATMENT EFFECTS IN HUMAN BREAST CANCER XENOGRAFTS USING MR IMAGING AND SPECTROSCOPY
456. Khalid S. Ibrahim: INTRAOPERATIVE ULTRASOUND ASSESSMENT IN CORONARY ARTERY BYPASS SURGERY – WITH SPECIAL REFERENCE TO CORONARY ANASTOMOSES AND THE ASCENDING AORTA
457. Bjørn Øglænd: ANTHROPOMETRY, BLOOD PRESSURE AND REPRODUCTIVE DEVELOPMENT IN ADOLESCENCE OF OFFSPRING OF MOTHERS WHO HAD PREECLAMPSIA IN PREGNANCY
458. John Olav Roaldset: RISK ASSESSMENT OF VIOLENT, SUICIDAL AND SELF-INJURIOUS BEHAVIOUR IN ACUTE PSYCHIATRY – A BIO-PSYCHO-SOCIAL APPROACH
459. Håvard Dalen: ECHOCARDIOGRAPHIC INDICES OF CARDIAC FUNCTION – NORMAL VALUES AND ASSOCIATIONS WITH CARDIAC RISK FACTORS IN A POPULATION FREE FROM CARDIOVASCULAR DISEASE, HYPERTENSION AND DIABETES: THE HUNT 3 STUDY
460. Beate André: CHANGE CAN BE CHALLENGING. INTRODUCTION TO CHANGES AND IMPLEMENTATION OF COMPUTERIZED TECHNOLOGY IN HEALTH CARE
461. Latha Nrugham: ASSOCIATES AND PREDICTORS OF ATTEMPTED SUICIDE AMONG DEPRESSED ADOLESCENTS – A 6-YEAR PROSPECTIVE STUDY

462. Håvard Bersås Nordgaard: TRANSIT-TIME FLOWMETRY AND WALL SHEAR STRESS ANALYSIS OF CORONARY ARTERY BYPASS GRAFTS – A CLINICAL AND EXPERIMENTAL STUDY

Cotutelle with University of Ghent: Abigail Emily Swillens: A MULTIPHYSICS MODEL FOR IMPROVING THE ULTRASONIC ASSESSMENT OF LARGE ARTERIES

2011

463. Marte Helene Bjørk: DO BRAIN RHYTHMS CHANGE BEFORE THE MIGRAINE ATTACK? A LONGITUDINAL CONTROLLED EEG STUDY
464. Carl-Jørgen Arum: A STUDY OF UROTHELIAL CARCINOMA: GENE EXPRESSION PROFILING, TUMORIGENESIS AND THERAPIES IN ORTHOTOPIC ANIMAL MODELS
465. Ingunn Harstad: TUBERCULOSIS INFECTION AND DISEASE AMONG ASYLUM SEEKERS IN NORWAY. SCREENING AND FOLLOW-UP IN PUBLIC HEALTH CARE
466. Leif Åge Strand: EPIDEMIOLOGICAL STUDIES AMONG ROYAL NORWEGIAN NAVY SERVICEMEN. COHORT ESTABLISHMENT, CANCER INCIDENCE AND CAUSE-SPECIFIC MORTALITY
467. Katrine Høyer Holgersen: SURVIVORS IN THEIR THIRD DECADE AFTER THE NORTH SEA OIL RIG DISASTER OF 1980. LONG-TERM PERSPECTIVES ON MENTAL HEALTH
468. MarianneWallenius: PREGNANCY RELATED ASPECTS OF CHRONIC INFLAMMATORY ARTHRITIDES: DISEASE ONSET POSTPARTUM, PREGNANCY OUTCOMES AND FERTILITY. DATA FROM A NORWEGIAN PATIENT REGISTRY LINKED TO THE MEDICAL BIRTH REGISTRY OF NORWAY
469. Ole Vegard Solberg: 3D ULTRASOUND AND NAVIGATION – APPLICATIONS IN LAPAROSCOPIC SURGERY
470. Inga Ekeberg Schjerve: EXERCISE-INDUCED IMPROVEMENT OF MAXIMAL OXYGEN UPTAKE AND ENDOTHELIAL FUNCTION IN OBESE AND OVERWEIGHT INDIVIDUALS ARE DEPENDENT ON EXERCISE-INTENSITY
471. Eva Veslemøy Tyldum: CARDIOVASCULAR FUNCTION IN PREECLAMPSIA – WITH REFERENCE TO ENDOTHELIAL FUNCTION, LEFT VENTRICULAR FUNCTION AND PRE-PREGNANCY PHYSICAL ACTIVITY
472. Benjamin Garzón Jiménez de Cisneros: CLINICAL APPLICATIONS OF MULTIMODAL MAGNETIC RESONANCE IMAGING
473. Halvard Knut Nilsen: ASSESSING CODEINE TREATMENT TO PATIENTS WITH CHRONIC NON-MALIGNANT PAIN: NEUROPSYCHOLOGICAL FUNCTIONING, DRIVING ABILITY AND WEANING
474. Eiliv Brenner: GLUTAMATE RELATED METABOLISM IN ANIMAL MODELS OF SCHIZOPHRENIA
475. Egil Jonsbu: CHEST PAIN AND PALPITATIONS IN A CARDIAC SETTING; PSYCHOLOGICAL FACTORS, OUTCOME AND TREATMENT
476. Mona Høysæter Fenstad: GENETIC SUSCEPTIBILITY TO PREECLAMPSIA : STUDIES ON THE NORD-TRØNDELAG HEALTH STUDY (HUNT) COHORT, AN AUSTRALIAN/NEW ZEALAND FAMILY COHORT AND DECIDUA BASALIS TISSUE
477. Svein Erik Gaustad: CARDIOVASCULAR CHANGES IN DIVING: FROM HUMAN RESPONSE TO CELL FUNCTION
478. Karin Torvik: PAIN AND QUALITY OF LIFE IN PATIENTS LIVING IN NURSING HOMES
479. Arne Solberg: OUTCOME ASSESSMENTS IN NON-METASTATIC PROSTATE CANCER
480. Henrik Sahlin Pettersen: CYTOTOXICITY AND REPAIR OF URACIL AND 5-FLUOROURACIL IN DNA
481. Pui-Lam Wong: PHYSICAL AND PHYSIOLOGICAL CAPACITY OF SOCCER PLAYERS: EFFECTS OF STRENGTH AND CONDITIONING
482. Ole Solheim: ULTRASOUND GUIDED SURGERY IN PATIENTS WITH INTRACRANIAL TUMOURS
483. Sten Roar Snare: QUANTITATIVE CARDIAC ANALYSIS ALGORITHMS FOR POCKET-SIZED ULTRASOUND DEVICES
484. Marit Skyrud Bratlie: LARGE-SCALE ANALYSIS OF ORTHOLOGS AND PARALOGS IN VIRUSES AND PROKARYOTES
485. Anne Elisabeth F. Isern: BREAST RECONSTRUCTION AFTER MASTECTOMY – RISK OF RECURRENCE AFTER DELAYED LARGE FLAP RECONSTRUCTION – AESTHETIC OUTCOME, PATIENT SATISFACTION, QUALITY OF LIFE AND SURGICAL RESULTS;

HISTOPATHOLOGICAL FINDINGS AND FOLLOW-UP AFTER PROPHYLACTIC MASTECTOMY IN HEREDITARY BREAST CANCER

486. Guro L. Andersen: CEREBRAL PALSY IN NORWAY – SUBTYPES, SEVERITY AND RISK FACTORS
487. Frode Kolstad: CERVICAL DISC DISEASE – BIOMECHANICAL ASPECTS
488. Bente Nordtug: CARING BURDEN OF COHABITANTS LIVING WITH PARTNERS SUFFERING FROM CHRONIC OBSTRUCTIVE PULMONARY DISEASE OR DEMENTIA
489. Mariann Gjervik Heldahl: EVALUATION OF NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER BASED ON MR METHODOLOGY
490. Lise Tevik Løvseth: THE SUBJECTIVE BURDEN OF CONFIDENTIALITY
491. Marie Hjelmseth Aune: INFLAMMATORY RESPONSES AGAINST GRAM NEGATIVE BACTERIA INDUCED BY TLR4 AND NLRP12
492. Tina Strømdal Wik: EXPERIMENTAL EVALUATION OF NEW CONCEPTS IN HIP ARTHROPLASTY
493. Solveig Sigurdardottir: CLINICAL ASPECTS OF CEREBRAL PALSY IN ICELAND. A POPULATION-BASED STUDY OF PRESCHOOL CHILDREN
494. Arne Reimers: CLINICAL PHARMACOKINETICS OF LAMOTRIGINE
495. Monica Wegling: KULTURMENNESKETS BYRDE OG SYKDOMMENS VELSIGNELSE. KAN MEDISINSK UTREDNING OG INTERVENSJON HA EN SELVSTENDIG FUNKSJON UAVHENGIG AV DET KURATIVE?
496. Silje Alvestad: ASTROCYTE-NEURON INTERACTIONS IN EXPERIMENTAL MESIAL TEMPORAL LOBE EPILEPSY – A STUDY OF UNDERLYING MECHANISMS AND POSSIBLE BIOMARKERS OF EPILEPTOGENESIS
497. Javaid Nauman: RESTING HEART RATE: A MATTER OF LIFE OR DEATH – PROSPECTIVE STUDIES OF RESTING HEART RATE AND CARDIOVASCULAR RISK (THE HUNT STUDY, NORWAY)
498. Thuy Nguyen: THE ROLE OF C-SRC TYROSINE KINASE IN ANTIVIRAL IMMUNE RESPONSES
499. Trine Naalsund Andreassen: PHARMACOKINETIC, PHARMACODYNAMIC AND PHARMACOGENETIC ASPECTS OF OXYCODONE TREATMENT IN CANCER PAIN
500. Eivor Alette Laugsand: SYMPTOMS IN PATIENTS RECEIVING OPIOIDS FOR CANCER PAIN – CLINICAL AND PHARMACOGENETIC ASPECTS
501. Dorthe Stensvold: PHYSICAL ACTIVITY, CARDIOVASCULAR HEALTH AND LONGEVITY IN PATIENTS WITH METABOLIC SYNDROME
502. Stian Thoresen Aspenes: PEAK OXYGEN UPTAKE AMONG HEALTHY ADULTS – CROSS-SECTIONAL DESCRIPTIONS AND PROSPECTIVE ANALYSES OF PEAK OXYGEN UPTAKE, PHYSICAL ACTIVITY AND CARDIOVASCULAR RISK FACTORS IN HEALTHY ADULTS (20-90 YEARS)
503. Reidar Alexander Vigen: PATHOBIOLOGY OF GASTRIC CARCINOIDS AND ADENOCARCINOMAS IN RODENT MODELS AND PATIENTS. STUDIES OF GASTROCYSTOPLASTY, GENDER-RELATED FACTORS, AND AUTOPHAGY
504. Halvard Høilund-Kaupang: MODELS AND METHODS FOR INVESTIGATION OF REVERBERATIONS IN NONLINEAR ULTRASOUND IMAGING
505. Audhild Løhre: WELLBEING AMONG SCHOOL CHILDREN IN GRADES 1-10: PROMOTING AND ADVERSE FACTORS
506. Torgrim Tandstad: VOX POPULI. POPULATION-BASED OUTCOME STUDIES IN TESTICULAR CANCER
507. Anna Brenne Grønskag: THE EPIDEMIOLOGY OF HIP FRACTURES AMONG ELDERLY WOMEN IN NORD-TRØNDELAG. HUNT 1995-97, THE NORD-TRØNDELAG HEALTH STUDY
508. Kari Ravndal Risnes: BIRTH SIZE AND ADULT MORTALITY: A SYSTEMATIC REVIEW AND A LONG-TERM FOLLOW-UP OF NEARLY 40 000 INDIVIDUALS BORN AT ST. OLAV UNIVERSITY HOSPITAL IN TRONDHEIM 1920-1960
509. Hans Jakob Bøe: LONG-TERM POSTTRAUMATIC STRESS AFTER DISASTER – A CONTROLLED STUDY OF SURVIVORS' HEALTH 27 YEARS AFTER THE CAPSIZED NORTH SEA OIL RIG
510. Cathrin Barbara Canto, Cotutelle with University of Amsterdam: LAYER SPECIFIC INTEGRATIVE PROPERTIES OF ENTORHINAL PRINCIPAL NEURONS
511. Ioanna Sandvig: THE ROLE OF OLFACTORY ENSHEATHING CELLS, MRI, AND BIOMATERIALS IN TRANSPLANT-MEDIATED CNS REPAIR

512. Karin Fahl Wader: HEPATOCYTE GROWTH FACTOR, C-MET AND SYNDECAN-1 IN MULTIPLE MYELOMA
513. Gerd Tranø: FAMILIAL COLORECTAL CANCER
514. Bjarte Bergstrøm: INNATE ANTIVIRAL IMMUNITY – MECHANISMS OF THE RIG-I-MEDIATED RESPONSE
515. Marie Søfteland Sandvei: INCIDENCE, MORTALITY, AND RISK FACTORS FOR ANEURYSMAL SUBARACHNOID HEMORRHAGE. PROSPECTIVE ANALYZES OF THE HUNT AND TROMSØ STUDIES
516. Mary-Elizabeth Bradley Eilertsen: CHILDREN AND ADOLESCENTS SURVIVING CANCER: PSYCHOSOCIAL HEALTH, QUALITY OF LIFE AND SOCIAL SUPPORT
517. Takaya Saito: COMPUTATIONAL ANALYSIS OF REGULATORY MECHANISM AND INTERACTIONS OF MICRORNAS

Godkjent for disputas, publisert post mortem: Eivind Jullumstrø: COLORECTAL CANCER AT LEVANGER HOSPITAL 1980-2004

518. Christian Gutvik: A PHYSIOLOGICAL APPROACH TO A NEW DECOMPRESSION ALGORITHM USING NONLINEAR MODEL PREDICTIVE CONTROL
519. Ola Storrø: MODIFICATION OF ADJUVANT RISK FACTOR BEHAVIOURS FOR ALLERGIC DISEASE AND ASSOCIATION BETWEEN EARLY GUT MICROBIOTA AND ATOPIC SENSITIZATION AND ECZEMA. EARLY LIFE EVENTS DEFINING THE FUTURE HEALTH OF OUR CHILDREN
520. Guro Fanneløb Giskeødegård: IDENTIFICATION AND CHARACTERIZATION OF PROGNOSTIC FACTORS IN BREAST CANCER USING MR METABOLOMICS
521. Gro Christine Christensen Løhaugen: BORN PRETERM WITH VERY LOW BIRTH WEIGHT – NEVER ENDING COGNITIVE CONSEQUENCES?
522. Sigrid Nakrem: MEASURING QUALITY OF CARE IN NURSING HOMES – WHAT MATTERS?
523. Brita Pukstad: CHARACTERIZATION OF INNATE INFLAMMATORY RESPONSES IN ACUTE AND CHRONIC WOUNDS

2012

524. Hans H. Wasmuth: ILEAL POUCHES
525. Inger Økland: BIASES IN SECOND-TRIMESTER ULTRASOUND DATING RELATED TO PREDICTION MODELS AND FETAL MEASUREMENTS
526. Bjørn Mørkedal: BLOOD PRESSURE, OBESITY, SERUM IRON AND LIPIDS AS RISK FACTORS OF ISCHAEMIC HEART DISEASE
527. Siver Andreas Moestue: MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF BREAST CANCER THROUGH A COMBINATION OF MR IMAGING, TRANSCRIPTOMICS AND METABOLOMICS
528. Guro Aune: CLINICAL, PATHOLOGICAL, AND MOLECULAR CLASSIFICATION OF OVARIAN CARCINOMA
529. Ingrid Alsos Lian: MECHANISMS INVOLVED IN THE PATHOGENESIS OF PRE-ECLAMPSIA AND FETAL GROWTH RESTRICTION. TRANSCRIPTIONAL ANALYSES OF PLACENTAL AND DECIDUAL TISSUE
530. Karin Solvang-Garten: X-RAY REPAIR CROSS-COMPLEMENTING PROTEIN 1 – THE ROLE AS A SCAFFOLD PROTEIN IN BASE EXCISION REPAIR AND SINGLE STRAND BREAK REPAIR
531. Toril Holien: BONE MORPHOGENETIC PROTEINS AND MYC IN MULTIPLE MYELOMA
532. Rooyen Mavenyengwa: *STREPTOCOCCUS AGALACTIAE* IN PREGNANT WOMEN IN ZIMBABWE: EPIDEMIOLOGY AND SEROTYPE MARKER CHARACTERISTICS
533. Tormod Rimehaug: EMOTIONAL DISTRESS AND PARENTING AMONG COMMUNITY AND CLINIC PARENTS
534. Maria Dung Cao: MR METABOLIC CHARACTERIZATION OF LOCALLY ADVANCED BREAST CANCER – TREATMENT EFFECTS AND PROGNOSIS