Exposure, Biochemistry and Health Risk of Aluminium in Breast Cancer: A Case-Control Study.

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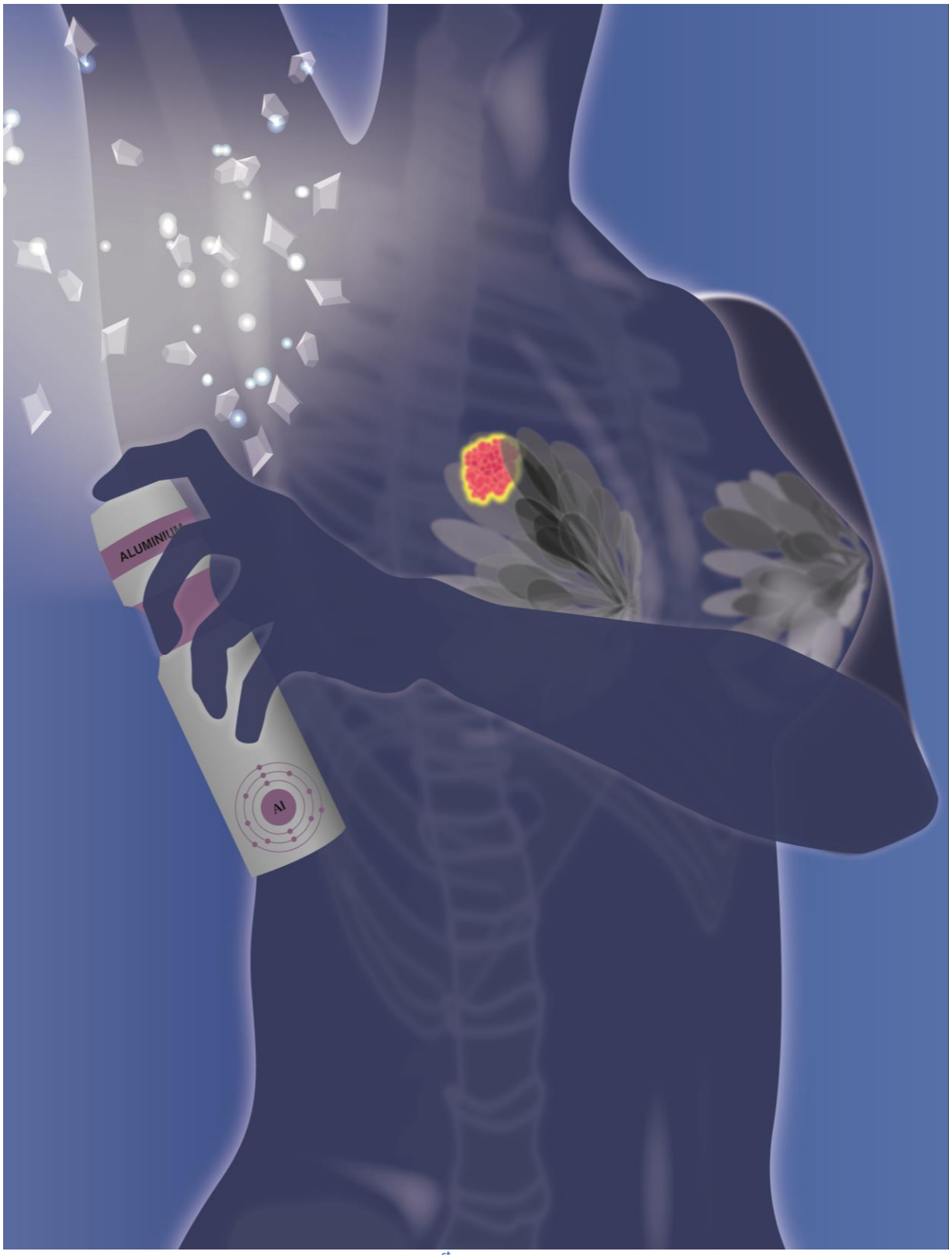

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Abstract

Background: The aetiology of breast cancer (BC) is multifactorial and presumably a combination of genetic, physiologic and environmental factors. Previous studies investigating the effect of underarm cosmetic products (UCP) containing aluminium (Al) salts on BC have shown conflicting results. Aluminium salts have long been associated with oxidative stress, proliferation, DNA double strand breaks and recently also with metastases. We conducted a 1:1 age-matched hospital-based case-control study aiming to investigate the risk for BC in relation to self-reported UCP application.

Methods: The study included a structured BC risk interview together with aluminium measurement in breast tissue. History of UCP use was compared between 209 female BC patients (cases) and 209 age-matched healthy controls. Aluminium concentration in tissue was measured in 100 cases and 52 controls that underwent either mastectomy or elective reduction mammoplasty. Additionally Al was analysed in blood and urine samples of 75 cases and 32 controls. Multivariable conditional logistic regression analysis was performed to determine relative risks, estimated as odds ratios (ORs) with 95% confidence intervals (CIs), adjusting for established BC risk factors.

Findings: Case-control comparisons confirmed established risk factors for BC including family history of BC, family history of other cancers and benign breast diseases. Self-reported use of UCP was significantly associated with an increased risk of BC ($p=0.036$). The risk for BC increased by an OR of 3.88 (95% CI 1.03-14.66) in women who reported using UCP's more than once a day when they were under the age of 30. More frequent UCP use was significantly associated with BC diagnosis at an earlier age ($p<0.001$). Aluminium in breast tissue was found in both cases and controls and was significantly associated to self-reported UCP use ($p=0.009$). Median (interquartile) aluminium concentrations were significantly higher ($p=0.001$) in cases than in controls (5.8, 2.3-12.9 versus 3.8, 2.5-5.8 nmol/g). Aluminium levels in urine and blood did not differ significantly from controls. Al levels in blood and tissue increased significantly ($p=0.034$, $p=0.032$) in relation to shaving of underarm hair. Results of self-reported physical exercise and Al levels suggest that regular exercise may reduce the body burden of Al.

Interpretation: Frequent use of UCPs may lead to an accumulation of aluminium in breast tissue and could lead to BC diagnosis at an earlier age. More than daily use of UCPs at younger ages may increase the risk of BC. Shaving of underarm hair may lead to higher Al levels while physical exercise seems to decrease the body burden of Al.

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Contents

List of abbreviations and acronyms

ACH	Aluminium chlorohydrate
AD	Alzheimer's disease
Al	Aluminium
Al^{3+}	free Aluminium ion
AlCl ($AlCl_3^+$)	Aluminium chloride
$AlCl_3^+$	Molecule of Aluminium chloride with loading
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
aq	aqueous
ATP	Adenosine triphosphate
BC	Breast cancer
BfR	Bundesinstitut für Risikoforschung
BMI	Body mass index
CI	Confidence interval
Crt	Creatinine
COPD	Chronic obstructive pulmonary disease
EFSA	European Food Safety Authority
GFAAS	Graphite furnace atomic absorption spectroscopy
HAS	Hydroxyaluminosilicates
HMM / HMW	High molecular mass / High molecular weight
IQR	Interquartile range
LMM/LMW	Low molecular mass / Low molecular weight
mRNA	messenger Ribonucleic acid
MS	Multiple sclerosis
NAF	Nipple aspirate fluid
NCD	Non-communicable diseases
OR	Odds ratio
PD	Parkinson disease
pH	potential of hydrogen
ROS	Reactive oxygen species
SD	Standard deviation
TWI	Tolerable weekly intake
UCP	Underarm cosmetic product

List of publications and presentations

Full paper

Linhart C, Talasz H, Morandi EM, Exley C, Lindner HH, Taucher S, Egle D, Hubalek, M, Concin, N, Ulmer, H. Use of Underarm Cosmetic Products in Relation to Risk of Breast Cancer: A Case-Control Study. *EBioMedicine* 2017; 21:79–85.

Abstract I

Linhart C, Talasz H, Morandi EM, Exley C, Lindner HH, Taucher S, Egle D, Hubalek, M, Concin, N, Ulmer, H. Use of underarm cosmetic products and breast cancer: a case-control study. (poster presentation at the International Meeting at the European Society of Gynaecological Oncology (ESGO), Vienna, 2017.)

Abstract II

Linhart C, Talasz H, Morandi EM, Exley C, Lindner HH, Concin N, Ulmer H. Breast cancer and the use of underarm hygiene products with aluminium-salts: A case-control study. *Keele Meeting* 2017; 12: 44-44 (oral presentation at the 12th Keele Meeting on Aluminium – Living in the Aluminium Age, Vancouver, 2017).

Abstract III (in German language)

Linhart C, Talasz H, Morandi EM, Exley C, Lindner HH, Concin N, Ulmer H. Brust – Antiperspirant aluminium salts and breast cancer: Preliminary data from a case control study. DGAUM 2016; 56: 26-26 (oral presentation at the 56. Wissenschaftliche Jahrestagung der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V., München, 2016.)

Abstract IV

Panosch D, Weidenbeck F, Hubalek M, Morandi E, Lindner H, Talasz H, Exley C, Concin N, Ulmer H, Linhart C. The use of antiperspirants containing aluminium-salts and its relation to breast cancer: Methods and implementation of bio-specimen sampling. *Keele Meeting* 2015; 11: 72-72 (poster at the 11th Keele Meeting on Aluminium – The Natural History of Aluminium. Past, Present and Future, Lille, 2015).

Abstract V

Linhart C, Kowalski J, Morandi EM, Lindner HH, Talasz H, Hubalek M, Exley C, Concin N, Ulmer H. Preliminary results and status of the study: The use of antiperspirants containing aluminium-salts and its relation to breast cancer. *Keele Meeting* 2015; 11: 47-47 (oral presentation and poster at the 11th Keele Meeting on Aluminium – The Natural History of Aluminium. Past, Present and Future, Lille, 2015).

Abstract VI

Linhart C, Concin N, Taucher S, Lindner HH, Ulmer H. Antiperspirants with aluminium-? salts and the relation to breast cancer. *Keele Meeting* 2013; 10: 83-83 (poster presentation at the 10th Keele Meeting on Aluminium, Winchester, 2013).

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1. Summary

The aetiology of most chronical, non-communicable diseases (NCDs) is multifactorial. Environmental, behavioural, metabolic and genetic risk factors contribute to the development of NCDs. Besides diabetes, cardiovascular and chronic respiratory diseases cancer is one of the main NCDs. While genetic factors are determined and behavioural and metabolic risk factors of diseases can be favoured or influenced, environmental factors are often too complex and dynamic to be overlooked and controlled. Air pollution and pesticides are well-studied and documented environmental risk factors, although the consequent risk management and prevention of their sources is still difficult. Additionally, there are sources of up to date barley ignored contaminants occurring either as pesticides from agricultural residues, or micro-plastics and nano-plastics from packaging or vast arrays of products in the form of additives in food, drugs, hygiene and cosmetic articles. Aluminium (Al) in is one of these chemicals especially in the form of Al salts. In vitro studies confirmed that Al is able to compete with other metals for binding sites of biological molecules, disrupt and break DNA double strands, to induce oxidative stress and proliferation. Depending on its exposure form and exposure time (acute- or long time) Al is linked to different NCDs. Recent studies investigated also the relationship of breast cancer (BC) and Al exposure. Al occurs as Al-chloride and Al-chlorohydrate in underarm cosmetic products like antiperspirants. Through its daily application and through skin damage occurring from underarm shaving the microenvironment of the breast is constantly exposed to an Al level beyond the tolerable weekly intakes stated by the EFSA (TWI 1 mg/kg body weight per week). On the one hand in-vitro studies and mouse models confirmed several exposure effects of Al in relation to BC: the over expression of mRNA S100 calcium binding proteins related to BC development, furthermore anchorage independent growth during in-vitro studies and increased metastases in mouse models support the hypothesis of Al exposure and BC. Al was also found in several biochemical analyses of breast tissue and nipple aspirate fluid derived from BC patients. On the other hand epidemiological studies did not reveal the exposure of Al through the use of antiperspirants as risk factor for BC. The conflicting results were rationale for conducting a comprehensive study with an epidemiological and biochemistry approach. A 1:1 age matched case-control study was conducted aiming to investigate the risk for BC in relation to self-reported underarm cosmetic product (UCP) use. The study included a structured BC risk

interview together with Al measurement in breast tissue of cancer patients and healthy individuals. Three tissue samples alongside the transect *axilla - mammillae - sternum* were sampled from 100 BC patients and 52 controls underwent either mastectomy or elective reduction mammoplasty. Furthermore 70 blood and urine samples of BC patients and 35 blood and urine samples of healthy individuals were collected. Tissue samples were weighted, dried and degreased to ensure standardized tissue samples with less fat content. All bio-samples underwent an acidic digest to analyse the samples as clear fluids with atomic-absorption spectroscopy (AAS). Data derived from the structured questionnaire concerning all established BC risk factors as well as nutrition, alcohol and the history of UCP use were compared between 209 female BC patients (cases) and 209 age-matched healthy controls. Therefore a multivariable conditional logistic regression analysis was performed to determine relative risks, estimated as odds ratios (ORs) with 95% confidence intervals (CIs), adjusting for established BC risk factors. Additionally, we correlated data derived from the questionnaire like self-reported history of UCP use with Al content in breast tissue of study participants. Case-control comparisons confirmed established risk factors for BC including family history of BC, family history of other cancers and benign breast disease.

Self-reported use of UCP was significantly associated with an increased risk of BC ($p=0.036$). The risk for BC increased by an OR of 3.88 (95% CI 1.03-14.66) in women who reported using UCPs more than once a day when they were under the age of 30. Al in breast tissue was found in both cases and controls and was significantly associated to self-reported UCP use ($p=0.009$). Median (interquartile) Al concentrations were significantly higher ($p=0.001$) in cases than in controls (5.8, 2.3-12.9 versus 3.8, 2.5-5.8 nmol/g). Al levels in urine and blood did not differ significantly from controls. Shaving of underarm hairs increased significant Al levels in blood ($p=0.034$) and tissue ($p=0.032$). Results of self-reported physical exercise were significant related to Al levels in blood and tissue of healthy subject ($p<0.05$) and suggest that regular exercise may reduce the body burden of Al. More frequent UCP use was significant associated to an earlier age of BC diagnosis ($p<0.001$). For the first time it is reported that the frequent use of UCPs may lead to an accumulation of Al in breast tissue and that especially the daily use of UCPs at younger ages increases the risk of BC. The study confirmed the uncontrolled results that more frequent use of UCPs may lead to BC diagnosis at an earlier age. While shaving of underarm hair may lead to higher Al levels, physical exercise, a protective factor for BC, seems to decrease the body burden of Al.

2. Zusammenfassung

Die Ätiologie von vielen chronischen, nicht übertragbaren Krankheiten ist multifaktoriell. Sowohl Umweltfaktoren, metabolische und genetische Risikofaktoren als auch individuelle Lebensweise und Verhalten wirken bei der Entstehung von chronischen Krankheiten zusammen. Neben Diabetes, kardiovaskulären und chronischen Atemwegserkrankungen zählt das Auftreten von bösartigen Tumoren zu den häufigsten chronischen, multifaktoriellen Erkrankungen. Während genetische Vorbelastungen determiniert sind, können metabolische Risikofaktoren durch individuelles Verhalten betreffend Ernährung und Lebensstil beeinflusst werden. Umweltfaktoren jedoch, sind oft zu komplex und dynamisch, um in ihrem ganzen Ausmaß und ihren Zusammenhängen erfasst zu werden, um wiederum mögliche Auswirkungen auf Umwelt und Gesundheit zu verhindern. Luftverschmutzung und diverse Pestizide gelten als Umweltrisiken, ihre Gesundheitsbelastungen sind bereits gut dokumentiert, auch wenn konsequente Vermeidung der Gefahrenquellen durch Verordnungen sich nach wie vor schwierig gestalten. Andere Umwelteinflüsse, die ein mögliches Gesundheitsrisiko bergen, wurden bisher großteils unterschätzt, wie etwa Pestizidrückstände aus der Landwirtschaft, Rückstände aus Verpackungen wie Mikro- und Nanoplastik sowie Zusatzstoffe in Nahrungs- und Arzneimitteln, Hygiene- und Kosmetikartikeln wie in Zahnpasta und Make-ups. Einer dieser Zusatzstoffe ist Aluminium (Al), besonders in Form von Al-Salzen. In-vitro Studien haben bereits das toxische Potential von Al aufgezeigt: Al konkurriert mit anderer Metallionen um die Bindung an biologisch aktive Moleküle und Al hat die Fähigkeit, DNA Doppelstrangbrüche zu initiieren, oxidativen Stress auszulösen sowie die Zellproliferation zu beschleunigen. Abhängig von der Expositionsform und Expositionsdauer (Akut-, Langzeitbelastung) wird Al mit verschiedenen chronischen Krankheiten in Verbindung gebracht. In den letzten Jahren wurde Al in mehreren Publikationen auch als Risikofaktor bei der Entstehung von Brustkrebs diskutiert. Al ist ein Bestandteil zahlreicher Kosmetik- und Hygieneartikel vor allem in Antitranspirantien wird es als Al-Salz (Al-Chlorid oder Al-Chlorohydrat) eingesetzt. Durch die tägliche Verwendung von Antitranspirantien und speziell durch zusätzliche Hautirritation, hervorgerufen durch das Rasieren der Achselhaare, ist die Umgebung der Brust dauerhaft einer erhöhten Al-Belastung ausgesetzt, die über der berechneten wöchentlichen Obergrenze der EFSA liegt (TWI 1 mg/kg Körpergewicht pro Woche). Die

Expositionseffekte von Al auf Brustkrebs wurden in mehreren In-vitro-Studien und vor kurzem auch in einem Mausmodell aufgezeigt. Es wurde eine Überexpression jener mRNA Abschnitte festgestellt, die vor allem die S100 kalziumbindenden Proteine kodieren und im Zusammenhang mit der Brustkrebsentwicklung stehen. Ebenso wurde eine deutliche Zunahme in der Zellproliferation sowie eine erhöhte Metastasierung im Mausmodell festgestellt. Diese Ergebnisse unterstützen den Verdacht, dass Al die Entstehung von Brustkrebs fördert. Ebenso wurden in malignem und benignem Brustgewebe als auch in der Brustflüssigkeit von Frauen, die an Brustkrebs leiden, erhöhte Al-Konzentrationen festgestellt. Der mögliche Zusammenhang von einer erhöhten Al-Exposition durch den Gebrauch von Antitranspirantien und der Entstehung von Brustkrebs wurde noch in keiner epidemiologischen Studie nachgewiesen. Diese widersprüchlichen Ergebnisse waren nun Anlass, eine umfassende epidemiologische Studie mit einem zusätzlichen biochemischen Ansatz durchzuführen. Um das Brustkrebsrisiko durch die Verwendung von Unterarmkosmetikprodukten, die vorwiegend Al-Salze beinhalten, zu prüfen, führten wir eine altersgepaarte Fall-Kontroll-Studie durch. Dazu wurden 209 an Brustkrebs erkrankte und 209 gesunde Frauen gebeten, an einem umfangreichen Interview teilzunehmen. Die gestellten Fragen betrafen allgemeine Brustkrebsrisiken, den Lebensstil der Frauen und im speziellen Hygienegewohnheiten. Zusätzlich wurden von 100 erkrankten Frauen, die sich einer Mastektomie unterziehen mussten, und 52 gesunden Frauen, die eine Brustverkleinerung vornehmen ließen, Brustgewebeproben entlang der Achse *Axilla – Mammilae – Sternum* entnommen und auf ihren Al-Gehalt untersucht. Ebenso wurden noch von 70 erkrankten Frauen und 35 gesunden Frauen der Al-Gehalt in Blut- und Urinproben gemessen. Um möglichst gleichmäßige Gewebeproben zu erhalten, wurden die Proben gewogen, getrocknet und entfettet. Alle Bioproben wurden einem Säureaufschluss unterzogen, um anschließend den Al-Gehalt in der klaren Flüssigkeit mittels der Atomabsorptionsspektrometrie (AAS) zu analysieren. Die gesammelten Interviewdaten von erkrankten und gesunden Frauen wurden bezüglich aller bereits bekannten Brustkrebsrisiken, der Ernährung, dem Alkoholkonsum, Lebensstil und der bisherigen Verwendung von Unterarmkosmetikartikeln miteinander verglichen. Für diese Gegenüberstellung und die weitere Risikoberechnung wurde eine multivariate konditionelle logistische Regressionsanalyse herangezogen. Das Risiko wurde mittels des Odds Ratios (OR) und des zugehörigen 95% Konfidenzintervalls angegeben und für bereits etablierte Brustkrebsrisiken adjustiert. Zusätzlich wurden Daten zur Anwendung

von Unterarmkosmetikprodukten mit dem gemessenen Al-Gehalt im Gewebe, im Blut und im Urin korreliert. Die Fall-Kontroll-Vergleiche der aufgenommenen Daten bestätigten etablierte Brustkrebsrisikofaktoren, wie familiäre Brustkrebsvorbelastung und Vorbelastung durch andere familiäre Krebsarten sowie vorangegangene benigne Brusterkrankungen. Die Interviewdaten zur Anwendung von Unterarmkosmetikprodukten stehen signifikant im Zusammenhang mit einem erhöhten Brustkrebsrisiko ($p=0.036$). Bei Frauen, die angaben, vor allem in jungen Jahren - unter 30 - mehrmals täglich ein Unterarmkosmetikprodukt angewendet zu haben, war das Brustkrebsrisiko mit einem Odds-ratio (OR) von 3.88 (95% CI 1.03-14.66), fast um das Vierfache erhöht. Ebenso zeigten die Resultate, dass eine vermehrte Anwendung von Deodorants/Antitranspirantien mit einer Brustkrebsdiagnose in einem früheren Alter einhergeht ($p<0.001$). Al wurde im Gewebe, Blut und Urin erkrankter und gesunder Frauen festgestellt. Al im Brustgewebe korrelierte signifikant mit der Häufigkeit der angegebenen Anwendung von Unterarmkosmetika ($p=0.009$). Mediane Al-Konzentrationen (Interquartil) waren signifikant höher ($p=0.001$) im Gewebe von Brustkrebspatientinnen als in gesunden Kontrollen (5.8, 2.3-12.9 versus 3.8, 2.5-5.8 nmol/g). Al-Gehalte in Urin und Blut unterschieden sich nicht signifikant zwischen gesunden und erkrankten Frauen. Während das Auftragen von Deodorants/ Antitranspirantien nach dem Rasieren der Achselhaare zu deutlich erhöhten Al-Werten in Blut und Gewebe beitragen kann ($p=0.034$, $p=0.032$), könnte durch regelmäßige körperliche Betätigung die Al-Belastung verringert werden. Allerdings wurde ein signifikanter Zusammenhang zwischen regelmäßiger körperlicher Betätigung und geringeren Al- Werten in Blut und Gewebe nur bei gesunden Frauen festgestellt.

Die an der medizinischen Universität durchgeführte Studie hat zum ersten Mal Al-Konzentrationen in Bioproben mit der Benutzung von Unterarmkosmetikprodukten korreliert. Es wurde erstmals festgestellt, dass ein häufiges Anwenden von Unterarmkosmetikprodukten zu einer Akkumulation von Al im Brustgewebe führen kann und dass vor allem eine mehrmals tägliche Anwendung in jüngeren Jahren zu einem erhöhten Brustkrebsrisiko beitragen kann. Die Studie bestätigte Resultate früherer unkontrollierter Studien, dass eine vermehrte Anwendung von Deodorants/Antitranspirantien möglicherweise mit einer Brustkrebsdiagnose in einem früheren Alter in Zusammenhang steht. Während das Rasieren der Unterarmhaare die Al-Belastung erhöht, könnte der protektive Brustkrebsfaktor, das regelmäßige Ausüben von Sport, auch die körperliche Gesamtbelastung von Al verringern.

3. Introduction

3.1 Environmental pollution and diseases

The aetiology of diseases is often multifactorial. Behavioural, environmental, metabolic and genetic risk factors contribute to the development of chronic non-communicable diseases (NCDs). The main types of NCDs are cardiovascular diseases, cancers, chronic respiratory diseases and diabetes[1,2].

While genetic factors are determined, behavioural and metabolic risk factors can be influenced, but environmental factors are often too complex and dynamic to be overlooked and managed by individuals. Modern medical research has two major battlefields: exploring the genome to optimize therapies on an individual level and investigating the exposome to gain information for disease prevention. Research on possible environmental factors like aluminium (Al) exposure and toxicology is an important contribution to disease prevention.

Environmental risk factors include exposure through environmental pollutions and occupational risks[3]. A pollutant usually is a chemical, ranging from simple ion (e.g., Al^{3+}) to complex organic molecules[4]. Worldwide about 8.9 million deaths per year are related to environmental pollution[5–7]. In contrast HIV/AIDS cause 1.5 million deaths per year and malaria and tuberculosis fewer than 1 million[5]. For example air pollution causes lung cancer, COPD, heart disease, stroke and respiratory diseases like asthma[7] and thus is most responsible for these deaths. Also contaminated soil at active and abandoned mines, smelters, industrial facilities and hazardous waste sites as well as pesticides used in agriculture like glyphosate are sources of environmental contaminants contributing very likely to NCDs like cancer, cerebrovascular diseases, neurodevelopmental disorders and birth defects in children[8]. It is still questionable if, how and to which extent various environmental hazards contribute to the development of NCDs. The reasons for these doubts are manifold. Once there are economic interests of various industries including not only the agricultural, mining and metal sector, the health sector has conflicting interests with disease prevention. Furthermore to receive clear results controlled and prospective study settings are the most important precondition to define and investigate the contribution of environmental hazards on the development of NDCs but this is due to the complexity of environmental factors very difficult, inherits always a bias and is cost and time intense. Concerning the complex

contribution of environmental hazards on NCDs and the Bradford Hill criteria are helpful and necessary to overcome bias and to provide epidemiologic evidence of a causal relationship[9]. Also new methodical approaches like mediation analysis are helpful to examine the complex contribution of environmental factors to the development of NCDs. To apply statistical methods like mediation analysis comprehensive, large and valid data sets as well as exact definitions and documentation of environmental risk factors are a basic requirement. However, sampling of valid data is cost and time intense.

More than 90% of pollution-related deaths occur in low-income and middle-income countries[10]. Toxic chemicals, hazardous pesticides and dangerous wastes from manufacture and recycling processes are often transferred from Western Europe and North America in low-income and middle-income countries like Africa, South Asia and Latin America and those countries are less equipped to deal with problems of pollution[10].

However ambient air pollution, new toxic chemicals and pesticides like glyphosate are still the predominant environmental hazards in richer countries. Many thousands of new chemicals have been invented in the past 50 years[6]. There are sources of up to date barley ignored contaminants occurring in a vast array of products in the form of additives in food, hygiene, cosmetic articles and drugs. Nowadays many chemicals occurring from these sources are detectable in the bodies of most people and many have never been adequately tested for safety[6,11]. And one of these chemicals is Aluminium (Al) in various compounds.

3.2 The third most abundant element: aluminium

Al is on earth the third most common element after oxygen and silicon[12] and the most abundant metal within the lithosphere[13]. Al is ubiquitous although it is a paradox that it has not any biological necessity[14]. The exposure to and the bioavailability of Al proceeded with environmental pollution increasing since post-industrial revolution. Over the last 200 years, mining, smelting and refining of Al in various forms have increasingly exposed living species to this naturally abundant metal[15]. In the last years Al is linked to a wide range of NCDs[13,16–18]. Depending on the exposure forms Al is related to Alzheimer's disease, dialysis dementia, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson disease, epilepsy, respiratory diseases[13,16–

20] and recently maybe also to breast cancer[21–25]. But why should in particular this common trivalent metal ion be under suspicion to be related to several diverse diseases like neurodegenerative diseases and cancer?

A possible explanation could be the role of Al during evolution and the changing biogeochemistry cycle through ongoing human intervention starting in the 19th century. Since the origin of life on earth, Al is stored as inert hydroxy-aluminosilicates (HAS) at neutral pH[14,16,20,26,27]. Through this strong binding with silica, Al was not active in biosphere and excluded from biochemical evolution[28].

Al is most common in the lithosphere where it's bound in ores like cryolite (aluminium fluoride) and bauxite (aluminium hydroxide). To extract Al from those ores the Bayer process, a highly energy-consuming process, is necessary. Therefore enormous hydro-electric power stations are built near Al plants, or vice versa. Pollution of water bodies near Al plants is possible and common.

The solubility of Al strongly depends on pH. At a lower pH (< 3.8), Al is released from minerals to soil solutions. (Figure 1 and Figure 2) Therefore, the slower process to leach Al from soil and its strong HAS is through decades of atmospheric pollution, starting with industrial evolution, leading to atmospheric acidification and acid rain and further to progressive acidification of soils, showing the peak level in the 1980ties.

Figure 1 The solution of Al3+ ions in water strongly depends on pH. Below the pH of 3.8 free aluminium cation occurs[12].

The low pH unlocked the strong silicate-aluminium bounding and Al distributed from earth crust to surface waters, especially in low buffered systems, with low calcium and magnesium amounts. (Figure 2) The lowered pH due to atmospheric pollution brings plants, animals and humans maybe for the first time in contact with the absorbable biologically reactive form of Al, the trivalent cation: $Al^{3+}_{(aq)}$[16,29,30]. (Figure 1) Aluminium binds to the extracellular matrix of apical root cells inhibiting the root development and elongation. Plants developed several mechanisms to defend aluminium toxicity, both Al accumulation and exclusion represent two co-occurring strategies[31,32]. Aluminium pollution in lakes was the reason for fish deaths during

1980ties in low buffered surface waters. Also the forest decline in granite areas like in parts of lower Austria, Czech Republic and Sweden occurred in the context of soil acidification and furthermore through related Al toxicity[12,33,34]. Al toxicity represents a serious problem for cultivation of agricultural plants on acidic soils and also for forest production.

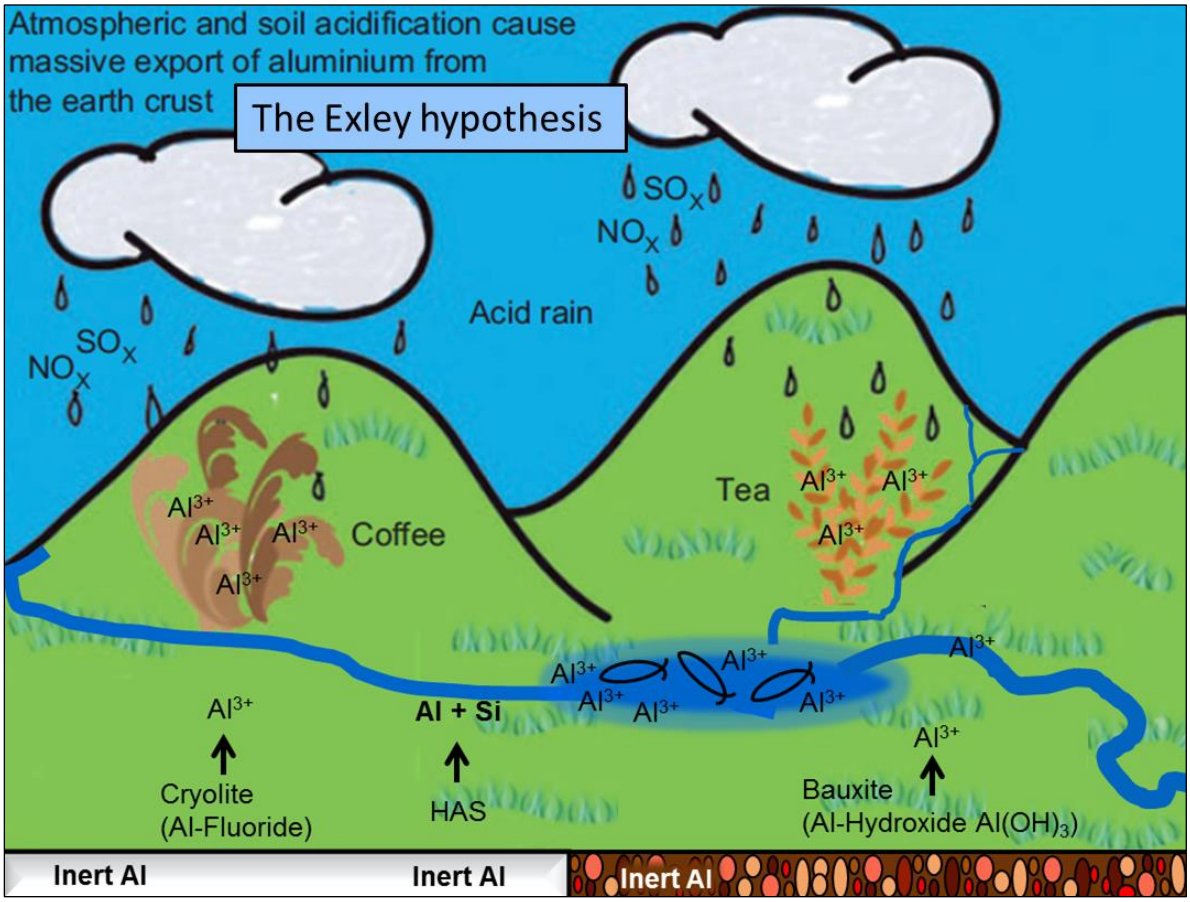

Figure 2 Al release from inert ores into the biosphere through acid rain caused by long lasting air pollution. Modified after Crisponi et al. (2013).

The terrestrial Al leaching persisted throughout the Holocene until the industrial period, afterwards the biologically available Al concentrations continuously increased[35,36].

4. The aluminium exposome

4.1 Aluminium exposure and uptake routes for humans

The geochemical cycle for Al has become a biogeochemical cycle, primarily due to interference of human activities. The most recent Al source is not only the industrial Al extraction from its biologically-inert ores through mining, smelting and refining or air pollution but also its further application in modern life: Al compounds are used as

antacids, vaccines, coating for pills, antiperspirants and food additives[15]. Aluminium salts are also widely used in water treatment as coagulants to reduce organic matter, turbidity and microorganisms. The process usually consists of addition of an Al salt (often sulphate) at optimum pH and dosage, followed by flocculation, sedimentation and filtration[37]. The widespread presence of bioavailable Al in the environment and the diverse use of Al in our daily life like in waste-water clearance, in cosmetic and pharmaceutical products and in food additives (Table 1) makes it nearly impossible to avoid Al exposure[30].

Tea plants and herbs like marjoram and thyme show a higher natural Al concentration, whereas Al concentrations in coffee and soy-based milk formulas are from industrial alimentary process. The natural Al content in alimentary products is negligible compared to the Al derived from preparation process, storage and packing. Al containers, Al foils and plastic containers with Al coating are sources for Al leaching in alimentary products like, milk, milk powder, cheese, juices and sugar and confectionary[16].

Food is one aluminium source contributing to the body burden of Al[30]. Cosmetics like powders, make-ups, lipsticks and every-day hygiene products like toothpaste and antiperspirants also contain Al salts (Table 1), which are soluble on skin surface or on any other body biofilms.

Table 1 Examples for aluminium exposure in humans[12,16].

Source	Amount	Unit
Natural sources	2 – 5	mg/day
Unprocessed food	0.1 – 7	mg/kg
Carrots	1.7	mg/kg
Drinking water	0.02-0.07	mg/L
Tee	4-10	mg/L
Cooked spinach	5	mg/200g
Food cooked in aluminium pots	0.2 – 125	mg/kg
Soy-based infant milk formulas	6 – 11	mg/kg
Beverages in aluminium cans	0.04 – 1.0	mg/L
Coffee from aluminium mocha	0.8 – 1.2	mg/cup
Parenteral nutrition solutions for adults	40 – 135	µg/L
Parenteral nutrition solutions for infants	10 – 270	µg/L
Antiperspirants	50 – 75	mg (daily exposure)
Antacids	35 – 200	mg/dose
Buffered aspirin	9 – 50	mg/dose
Antidiarrheal drugs	36 – 1450	mg/dose
Food additives	10 – 20	mg/day
Vaccines	0.125 – 0.85	mg/dose

Depending on the various exposure possibilities (nutrition, aerosols, topical applications, vaccination) and uptake routes (gastrointestinal tract, inhalation, dermal absorption, muscle depots) of the specific Al compound, Al may be associated with different diseases. The uptake route of aluminium determines also the site of action; for nutrition and drugs it's the gut, for vaccines mainly muscle tissue, for cosmetics, crèmes and antiperspirants the skin and for aerosols and sprays the nose and the lung. Therefore the health risk of Al strongly depends on the interaction and storage site (whether blood, brain, tissue or bones) which are in turn determined by the resorption of Al via various exposure and uptake routes[30,38]. (Figure 3)

Acute Al toxicity is rather uncommon in clinical practice but occurred in renal-failure patients leading to osteomalacia and dementia[39–42]. A neurological syndrome, either called "dialysis encephalopathy" or "dialysis dementia" occurred in dialysis patients treated with oral medications for phosphate-binding to control hyperphosphatemia, containing Al. Foremost patients with chronic renal failure were exposed to Al, either by oral medication, by domestic tap-water supplies used either for drinking or, in those on dialysis treatment, in the preparation of their dialysate[43]. Other people exposed to very high Al levels via contaminated water, as occurred in Camelford in the late 1980ties due to contamination of the local water supply with enormous amounts of Al, suffered and died of a rapid and abrasive forms of dementia. Higher Al levels were found in bone, brain, and other tissues of those dementia patients former exposed to Al[44–46]. Al is associated with toxic secondary disorders and an increased brain content of Al appears to be an etiological factor in the development of dementia[39,40]. Patients with Alzheimer's disease showed in specific brain regions higher Al levels especially in plaques consisting of β-amyloid-proteins[44,47]. Dialysis encephalopathy, as occurred in patients with renal failure, is well studied, but little is known about repeated long term low level Al exposure through various exposure and action sites.

Furthermore it is reported that mining workers who had to inhale Al dust before starting their daily work, developed other neurodegenerative diseases like Parkinson disease (PD) and Alzheimer's disease[48–51]. In the substantia nigra of patients suffering from PD also higher Al levels were measured[52–56].

Due to unclear mechanisms how Al induces or contributes to various NCDs, it is still unclear and discussed if Al is accumulating in affected people as side effect or if Al itself contributes to disease development. People affected either with PD, MS or forms of

dementia, reported higher Al exposure during their life-time and measured Al levels in their bio-samples were higher. But recent publications including controlled, prospective studies are lacking.

A structured literature search for full articles (excluding comments) on PubMed for Al and diseases in high impact factor journals was therefore conducted. Following search terms were used for the four different journals:

```
("Lancet"[Journal]
OR "Lancet"[All Fields]
OR "N Engl J Med"[Journal]
OR "Nature"[Journal]
OR "Science"[Journal])
AND ("aluminium"[All Fields]
OR "aluminum"[All Fields]
AND "disease"[All Fields]
```

The results showed that Al was a focused research topic and highlighted as serious environmental factor for NCD. (Table 2) Most studies were published during the 1980ties, but during the 1990ties the disease related topics around Al disappeared from the high impact journals. Interestingly, around 2010 Al returned in studies about HPV vaccine trials. *The Lancet* published most studies about Al related health risks, AD and dementia and also about bladder and colon cancer. In the late 1990ties studies on Al disappeared for years. In the year 2006 Al related topics emerged again in *The Lancet* with focus on Al hydroxide as adjuvant in vaccines. An extended search term[1] showed that between 2006 and 2017 the selected four journals published 20 full articles containing "aluminium" only mentioned as adjuvant in relation to vaccine studies, but not as independent research topic.

Table 2: Results of PubMed search for aluminium and diseases presented as absolute numbers of publications and the year of the last publication in brackets.

Subject	Science	Nature	N Engl J Med	Lancet
Aluminium/Aluminum				209 (1999)
Aluminium + disease	4 (1995)	10 (1993)	13 (1991)	47 (1999)
Aluminium + Alzheimer + dementia	4 (1995)	0	4	31 (1999)
Aluminium + cancer	0	0	0	8 (1984)

In general most studies on Al exposure are focusing on Al exposure via nutrition[57–61]. Al occurs foremost as food additive, like bentonite (E 558), calcium-aluminium-sulphate (E 556) and caolin (E 559). The European Food Safety Authority (EFSA) calculated a

[1] ("Lancet"[Journal] OR "lancet"[All Fields] OR "N Engl J Med"[Journal] OR "Nature"[Journal] OR "Science"[Journal]) AND ("aluminium"[All Fields] OR "aluminum"[All Fields] OR "alum"[All Fields]) AND ("disease"[All Fields] OR "safety"[All Fields] OR "risk"[All Fields])

mean bioavailability of 0.1% for all Al compounds derived from nutrition, which is 0.143 µg/kg body mass for a tolerable daily systemic uptake[59,62]. Therefore the daily systemic doses without health risk would be 8.6 µg per day for an adult of 60 kg.

Regarding the oral resorption of Al from nutrition the Joint FAO/WHO Expert Committee on Food Additives specified a new tolerable weekly intake (TWI) on Al and reduced the TWI from 7 mg Al/kg body weight to 1 mg Al/kg body weight, which is still routinely exceeded especially by children. Children have a higher food intake than adults when expressed on body weight basis and have the highest potential exposure to aluminium per kg body weight. Furthermore potential dietary exposures from infant formulae and other foods manufactured specially for infants were estimated from 0.10-0.78 mg/kg body weight per week[59]. Since 2014 the EFSA restricted some Al food additives[61].

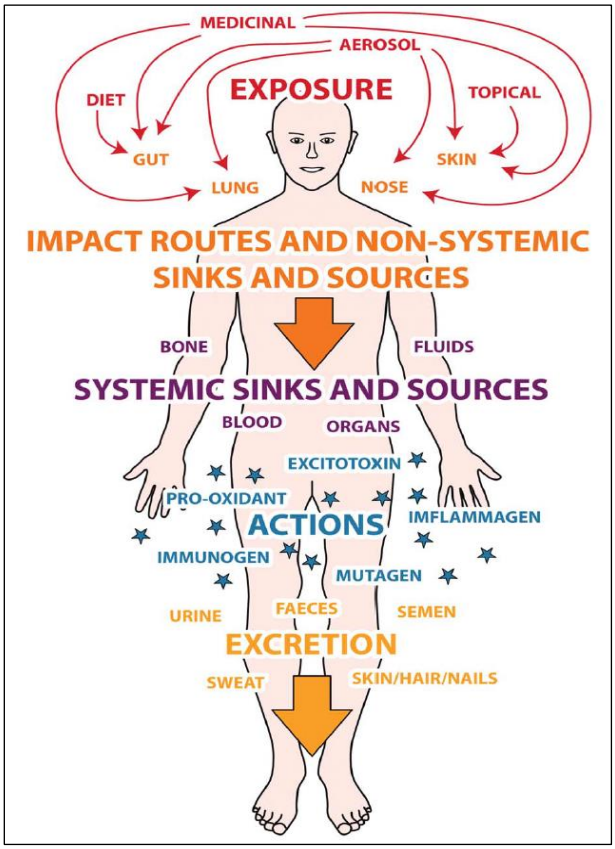

Figure 3 Aluminium's exposome after Exley (2013). The Figure describes the relation between Al exposure, immediate targets mediating exposure sites and sinks of biologically available aluminium with assumed mechanisms of action and finally excretion of aluminium[30]

According to the Table 1, Crisponi et al. (2013) stated that antiperspirants are one of the major Al exposure sources. Antiperspirants are a subgroup of deodorants that affect odour as well as prevent sweating by blocking the sweat glands. Deodorants, commonly

termed '*deo*', are products containing alcohols, glycols and fragrances, intended to reduce body odour, but normally contain no active products holding back the release of sweat. Antiperspirants active substances are mostly Al salts like Aluminium Chlorohydrate (ACH, $Al_nCl_{(3n-m)}(OH)_m$) or Aluminium Chloride ($AlCl_3$)[63]. The distinction between antiperspirants and deodorants is not always clearly for consumers. In Europe, deodorants are more popular, but the use of antiperspirants was rising until the last years[64]. In the US more than 90% of the adult consumers use an antiperspirant or a deodorant regularly. Many forms exist: gels, sticks, roll-ons and sprays and antiperspirants and deodorants have grown to be the largest health and beauty aids product categories in the US[65].

Crisponi et al. (2013) mentioned per topical application of antiperspirants on the axilla an Al exposure of 50-75 mg. Despite the dose it's still unclear how much Al is absorbed through the skin. The ability of dermal absorption of Al from UCP use was reported in a clinical case study[66]. The report described Al uptake from UCP application in a women up to a toxic level of 4 µM in blood plasma in relation to symptoms of bone pain and fatigue. After cessation of UCP use, symptoms disappeared and Al levels downsized to normal range (0.1-0.3 µM)[66]. The report strongly indicates that the Al levels in plasma and the associated symptoms resulted from UCP use[67]. Up to now just one study with only one female and one male participant[68] calculated the dermal absorption of about 4 µg (*0.012% of aluminium from antiperspirant exposure)* from one single application of an Al chlorohydrate (ACH) containing underarm cosmetic product (UCP). The revisions of studies[68,69] on aluminium absorption from antiperspirant application by the German Federal Institute of Risk Assessment (BfR) resulted in a calculated systemic resorption around 10.5 µg, higher than the calculated limit of daily systematic uptake from nutrition specified through the TWI by EFSA (8.6 µg/day)[70]. Values for shaved or injured skin would be even higher. According to these studies and reports the daily use of an antiperspirant would exhaust the TWI and an accumulation of Al in the human body would be very likely. Studies on chronic low level Al exposure via the skin through UCPs are lacking and – despite reasonable suspicion - the relation of Al exposure, its uptake and the mechanism leading to possible health risks are still not clear.

4.1.1 Aluminium and the microenvironment of the breast

Deodorants and antiperspirants are applied mainly under the armpit, in the axilla. Some women reported also to use UCPs in addition under their breasts. Through repeated application of UCPs containing Al salts the microenvironment of the breast is continuously exposed to Al. The extent of Al exposure to the breast microenvironment and further the possible role of Al in cancer development are still unclear. A change in the topological distribution of mammary carcinoma since 1975[71–76] towards a higher incidence in the upper outer quadrant seems to point to UCPs as a potential contributor[73,74,76,77]. Several studies measured Al levels in breast structures of breast cancer patients showing that the intraductal microenvironment of the breast is exposed to Al. Levels vary regarding measurement method, however modern and standardized measurement techniques show mean levels for breast tissue between 0.25 and 2 µg/g[78,79]. Even samples of the same individuals show a high variation, assuming a patchy distribution of incorporated Al in tissue. In nipple aspirate fluid (NAF) Al levels were found to be around 100 to 300 µg/L, and were significant higher in women with breast cancer. Although, Al is measured in breast structures, it is unclear how Al is able to enter the human skin, how Al accumulates and interferes with essential ligands or disrupts biological pathways. Therefore the following sections should give an overview of recent concepts about the mechanisms of antiperspirants, the absorption of Al via the skin, the Al transportation in human body, its storage and excretion, leading to the hypothesis of Al's involvement in breast cancer development.

4.1.2 Mechanism of antiperspirants and aluminium absorption

The axilla has with approximately 25 000 eccrine sweat glands the highest density of sweat glands. The eccrine sweat glands are present on almost the whole body and they have the function to keep a constant body temperature of approximately 37°C[65]. Sweat consists of a watery electrolyte solution and contains chloride, potassium, ammonia and bicarbonate (Table 3). The pH of sweat is normally acidic around a pH of 5.4 but depending on the extent of sweat and the amount of bicarbonate pH can rise up to a level of eight[80].

Table 3 Human eccrine sweat composition

Organic compounds	Inorganic compounds
Acetic acid	Calcium Ca^{2+}
Ascorbic acid	Magnesium Mg^{2+}
Caprionic acid	Copper Cu^{2+}
Citric acid	Iron Fe^{3+}
Butyric acid	Kalium, K^{+}
Caprylic acid	Natriumchloride, NaCl
Formic acid	
Lactic acid	
Propionic acid	
Urea	
Ammonia	

The recent concept of antiperspirants mechanism assumes the precipitation of ACH inside the eccrine sweat glands. Through low pH, insoluble Al hydroxide is produced, which plugs together with dead cells of the stratum corneum and divers proteins the sweat gland. Thereby the secretion of sweat is blocked[22,81,82]. Other concepts discuss the active inhibition of sweat gland activity by the free Al^{3+} ion, favouring the assumption that the effect of antiperspirants is active rather than passive via gland plugging[83].

The clotting of the sweat glands is a similar process like the clotting of salmon gills occurring in acidic lakes with dissolved Al. Mechanisms of acute Al toxicity in fish was discussed intensely[14,84] and two different mechanisms are investigated: first, the mechanistic clotting of the gill surface through precipitation and Al polymerization of Al and second the binding of $Al^{3+}_{(aq)}$ by functional groups apically located at the gill surface and intracellularly within epithelial cells disrupting the barrier of the gill epithelium. The associated iono- and osmoregulatory dysfunction resulted in accelerated cell necrosis, sloughing and death of the fish. The mechanism of epithelial cell death was proposed as a general mechanism of aluminium-induced accelerated cell death[14]. Between pH 5 and 6 Al toxicity is most severe and a rapid rise in pH substantially increases the ongoing Al polymerization and the acute Al toxicity to fish[84–86]. A co-occurrence of both mechanisms is most likely.

It was also observed that AlCl can be absorbed through the skin of mice[87]. Of course human skin has about 20 to 30 cell layers and is thicker than the skin of mice and is even more different from fish gills. However it is proposed that long-term blockage of sweat

glands by frequent application of antiperspirants could also lead to secretory cell damage[64,88]. It's still not clear if the human skin is an effective barrier to transdermal uptake of Al (Figure 4). So far the BfR reviewed existing studies, including one in vivo study and one in-vitro study[68,69]. The only in-vivo study was rather a case study including two participants[68], a man and a women and measured urinary excretion of Al, while possible Al accumulation through long term and repeated application was neglected. Interestingly, the woman showed compared to the man less excretion of Al and higher dermal absorption. As mentioned by Crisponi et al. (2013) [16] the daily exposure of antiperspirants is about 50-75 mg (Table 1) by a single application and Flarend et al. (2001) calculated the 0.012% of Al absorption according to an Al amount of 84 mg per product application[68]. This would still result in an absorption for a single application of 0.008-0.03 µg/g in breast tissue, assuming a mean breast weight of 500g.

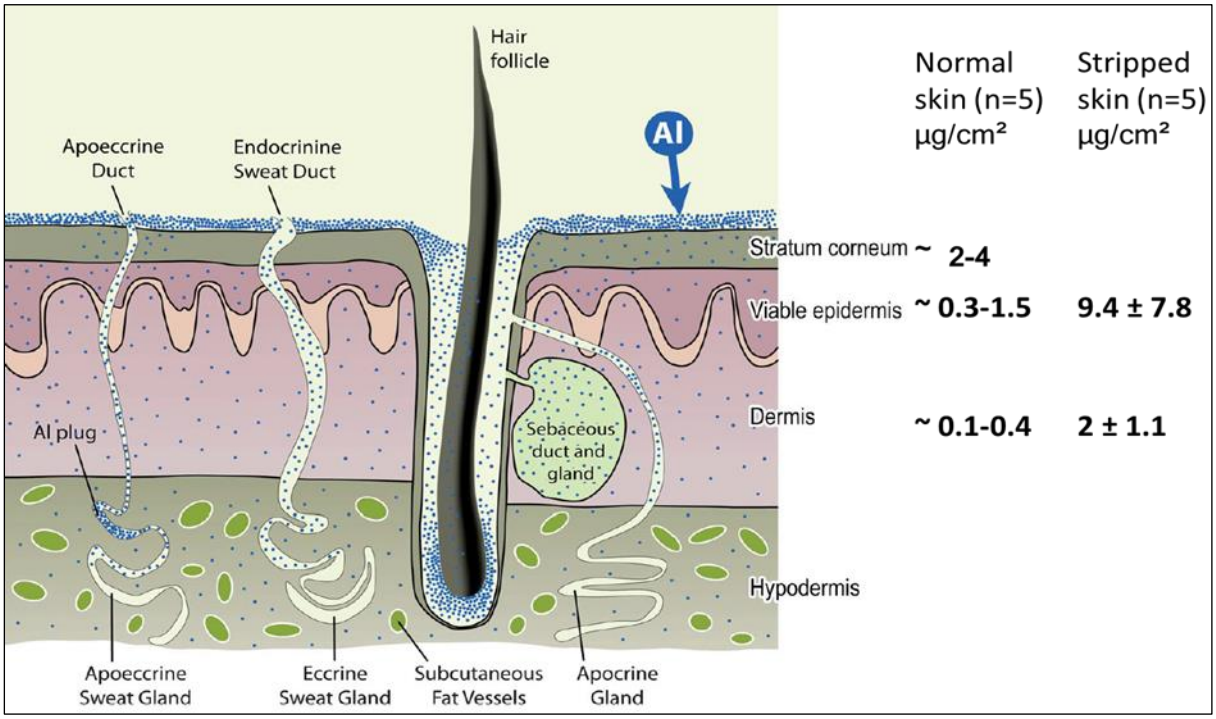

Figure 4 The skin is a sink for topically applied Al and will act as a source of biologically reactive Al both to structures within the skin and to the systemic circulation[30]. **Graphic according to Exley (2013) with additional data from Pineau (2012)**

Pineau et al., (2012) showed a significant difference in absorption levels of Al from antiperspirants between stripped (11.5 µg/cm^2) and normal skin (1.8 µg/cm^2). The study showed also different absorption rates for different antiperspirant application forms as sprays and sticks. The kinetics of Al transfer from percutaneous UCP application towards the blood pool is conditioned by a various number of factors that

may be cosmetic-dependent (pH, Al-compound) and tissue-dependent (thickness, integrity)[69].

In summary, it can be proposed that Al is absorbed through the skin, especially when the skin as an erased stratum corneum through shaving. Al accumulates through topical application in apoeccrine and endocrinine sweat ducts, polymerizes, forms plugs but also dissociates through pH changes. (Figure 4) Al is able to enter the skin in form of $AlCl_3$, ACH or as $Al^{3+}_{(aq)}$ alongside the sweat ducts or hair follicles or directly through micro injuries from shaving into deeper layers of the dermis. Aluminium's transfer through human skins and further kinetics in the body strongly depends on Al chemistry and it's complexation with possible ligands.

4.2 Transport and impact of aluminium on the cellular level

The transportation of Al across cell membranes is possible as $Al^{3+}_{(aq)}$ free cation or as charged or neutral complex. Exley (2014) describes five different forms of Al and five different routes across cell membranes respectively epi- or endothelial cells (Figure 5). The free solvated trivalent cation ($Al^{3+}_{(aq)}$) is mainly transported via para-cellular transport. Al has the ability to form various strong complexes with different bio-ligands, including low molecular mass (LMM) molecules such as citrate and high molecular mass (HMM) proteins such as transferrin[90]. Low-molar-mass, neutral and soluble complexes (LMW-$Al^0_{(aq)}$) are transported via trans-cellular diffusion, high-molar-mass, neutral and soluble complexes (HMW-$Al^0_{(aq)}$) via active transport, low-molar-mass, charged and soluble complexes (LMW-$Al(L)_n^{+/-}{}_{(aq)}$) mainly via channels and nano- and micro particles ($Al\ (L)_{n(s)}$) are transported via endocytosis inside the cell. (Figure 5) Total estimated Al values in the human body are around 50-150 mg, from this amount 40% should occur in the lungs, 25% in muscles, 25% in bones ad 10% in blood and brain. In muscles, liver and bones 1-5 mg/kg and in brain 2 mg/kg Al were determined[12]. Moreover Al is found in all body fluids: in blood, (plasma and serum <1µg/L), in cerebral spinal fluid, in interstitial fluid of the brain, in sweat, lymph, urine and semen. Aluminium's presence in systemic compartments shows its tendency to pass through epi-/endothelia or trans-cellular routes[30].

On the body surface, including skin and hair, measured aluminium concentrations are around 14-37 µg/kg. Furthermore Al is also found in breast milk with a concentration around 9.2 µg/L[12].

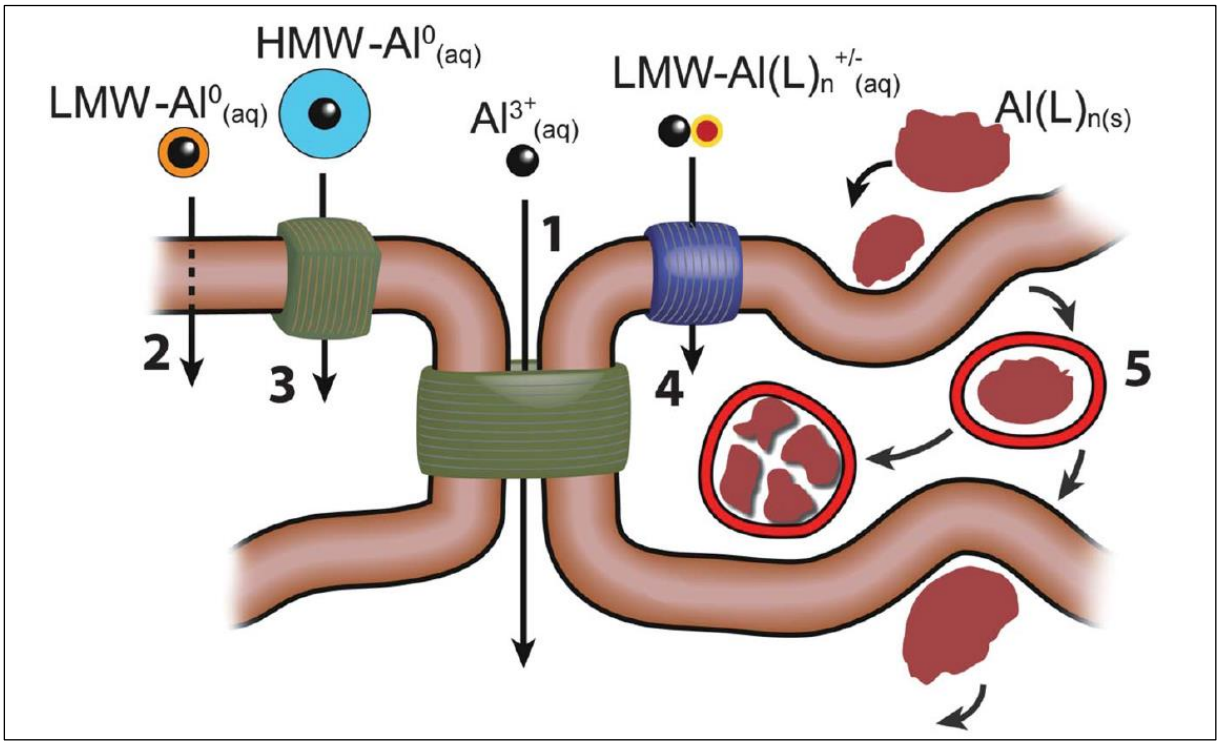

Figure 5 Major epi- or endothelial cell transportation routes for Al forms: para-cellular (1), trans-cellular diffusion (2), active transport (3), channels (4) and adsorptive or receptor mediated endocytosis (5)[30].

In the case of Al exposure through antiperspirant application, Al passes the stratum corneum and the viable dermis through dermis and hypodermis likely as trivalent cation. Through small injuries resulting from underarm hair shaving, Al and its complexes would reach directly the blood and later the lymph stream and if not eliminated through sweat and urinary excretion it would reach also tissue, bone and brain structures. In these structures, Al could accumulate, and locked away in inert precipitates or otherwise it could be highly reactive with several ligands disrupting important biological mechanisms.

Although the exact mechanism of Al toxicity is still not clear, it is known that the chemical reactivity of Al, the solubility and hydrolysis and further its toxicity is different for various Al species in solution, and depends on pH, temperature and the presence of other inorganic (F^-, OH^-, PO_4^{3-}) and organic ligands (citric acid, ferritin, transferrin)[84].

In blood, 80% of Al is bound to transferrin, especially HMM Al species. Few amounts of serum Al may also bind to albumin[91]. Al species with LMM are bound to oxygen-donor ligands like phosphate (16% of serum Al) or citrate (1.9 % of serum Al). Only 0.8 % occur as $Al(OH)_3$ und 0.6 % occur as $[Al(OH)_4]^-$ and small amounts may occur as free solvent cation $Al^{3+}{}_{(aq)}$[12,91,92]. The Al species, low or high molecular mass or free cation, is dominated by pH and as mentioned before Al is primary biological reactive in

the form of its free, solvates trivalent cation $Al^{3+}_{(aq)}$. At lower pH values (4.2-5), the amount of mobile and reactive free solvent $Al^{3+}_{(aq)}$ cations would rise[12]. (Figure 1)

Other mentioned bioligands for Al(III) are silicic acid, lactic acid, oxalic acid, catecholamines, ATP and hydroxide[15,91].

There are still uncertainties regarding the stability constants of the LMM Al complexes and the complexity in the aqueous chemistry of Al effected also Al toxicity studies[91]. But it is for sure that Al is highly reactive with biomolecules and that it builds strong bondings that are extremely slow to dissociate[89,93,94]. Therefore it could be either locked away in complexes, polymers or precipitates and is able to accumulate in bone and tissue or Al interacts with various bio-ligands on the molecular and protein level.

Al can interact with the side chains of some phosphorylated, amino acids like serine, threonine and tyrosine, disrupt the side chains and involved processes[15,95,96]. Al is also able to interfere with sulphur containing amino acids like cysteine, methionine and glutathione, because of the strong binding affinity of Al to sulphur oxyanions[15], all involved in methylation and transculturation processes. Al competes effectively with essential metals, in particular it's a mmajor antagonist of magnesium (Mg^{2+}) and competitor for Ca^{2+} and Fe^{2+}. It is also likely that Al replaces magnesium in nucleotides[15] and in the catalytic sites of regulatory enzymes[15,97–100]. It competes with magnesium as a metal for the ATP co-factor and builds complexes with ATP[15,91]. Due to its same charge and similar ionic radii with ferric iron Al may mimic iron and bindto the iron transport protein, transferrin[101] and to the iron regulatory protein mRNA[102]. The conesquence is a disruption of the iron metabolism[90], promoting redox cycling and free radical formation[103]. Al as free solvent cation Al^{3+}(aq) has a high pro-oxidant activity. It forms ligands mainly with oxygen donors. (Fig. 5) The oxidant activity of Al works through the formation of an Al-superoxide ($O_2^{\bullet -}$ radical) complex. (Figure 6)

The Fenton reaction is the main source of reactive oxygen species (ROS) in biological systems leading to oxidative stress in the cell. Confirmed redox reactions are found for AlO^{2+}, $Al(OH)(O^{\bullet})^{+}$ and Al(OH) ($O^{\bullet}$), all Al-superoxide species[90]. (Figure 7)

Free radical formation and chronic oxidative stress subsequently contribute to tumour initiation and tumour growth[103]. By several variations of the Ames test, one of the

most common biological assays used in the industry to assess the mutagenic potential of chemical compounds, Al was not detectably mutagenic in bacteria[24]. Despite the pro-inflammatory and pro-oxidative abilities of Al, the continuous exposure of the breast microenvironment and the measured Al levels in breast structures, Al is still discussed as a breast carcinogen.

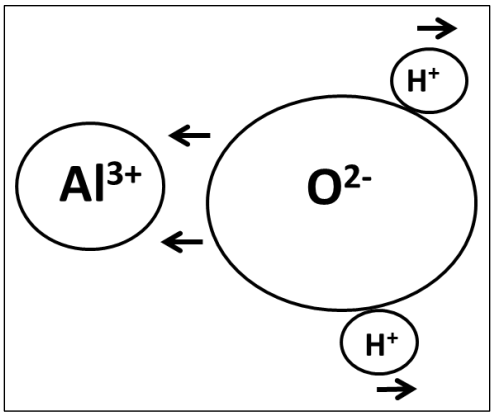

Figure 6 The acid reaction of the Al ion in water: The binding of H and O is disrupted through the strong positive loading of Al^{3+} and H^+ dissociates because of the negative charged oxygen. Through the H^+ dissociation and its transfer to water, H_3O^+ is formed and the acid reaction starts. The other proton is not dissociating. The ambition of the H_2O molecule in the hydrate of the Al^{3+}(aq) complex is to get rid of the protons[12].

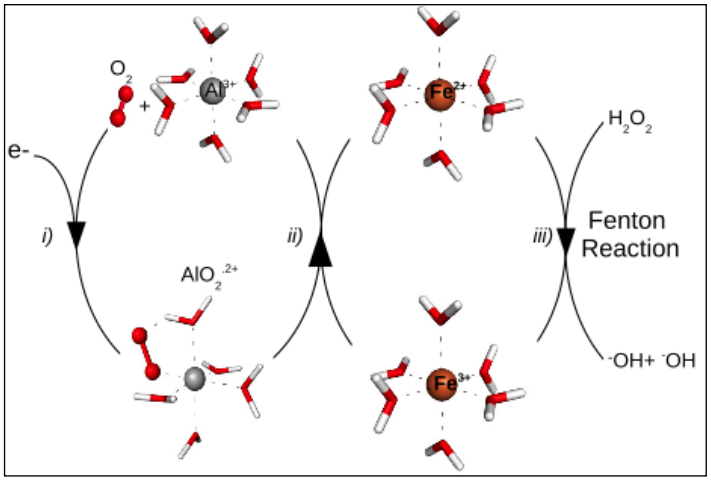

Figure 7 Al^{3+} Fenton promotion cycle: i) Al^{3+} stabilizes and forms an Al-superoxide complex; ii) Al-superoxide reduces Fe^{3+} to Fe^{2+}, leading to a spontaneous dissociation of oxygen from Al and recovering the initial Al^{3+} species; iii) Fe^{2+} oxidizes again to Fe^{3+} by the Fenton reaction, inducing the formation of radicals (·OH) that causes oxidative damage. At the end of the process, the initial Al^{3+} and Fe^{3+} species are recovered[90].

4.3 Aluminium and breast cancer: possible mechanism

Similar to an activated oncogene, Al induces proliferation-stress, DNA-double-strand breaks (DSB) and senescence in normal mammary epithelial cells. Cells with a long-term exposure to $AlCl_3$ generate an ability to bypass p53/p21Waf1-mediated cellular senescence[24]. Al concentration up to 100 000-fold lower than those found in antiperspirants, and in the range of those recently measured in the human breast, resulted in loss of contact inhibition and anchorage-independent growth. $AlCl_3$ also induced DSBs and senescence in proliferating primary human mammary epithelial cells[24].

In vivo experiments have shown that Al is able to interfere with oestrogen in the form of Al salts as occurring in antiperspirants. Al chloride and Al chlorohydrate have a metalloestrogen function interfering with oestrogen receptors of MCF7 human breast

cancer cells, both in terms of ligand binding and in terms of oestrogen-regulated reporter gene expression[74].

Furthermore, the exposure to Al can also increase migratory and invasive properties of MCF-7 human breast cancer cells, what suggests that the presence of Al in the human breast could influence metastatic processes[67,72]. This hypothesis was recently confirmed by Mandriota et al., (2016) showing that concentrations of Al in the range of those measured in the human breast first transform cultured mammary epithelial cells, and then if injected into different mouse strains enable them to form tumours and metastasize[23]. The observations of mouse cancer models provide experimental evidence that Al salts could be environmental breast carcinogens. Furthermore $AlCl_3$ treated cells show mutations of genes regulating cellular proliferation, migration, metastasis and apoptosis. The mutations also affected T-lymphoma invasion and a metastasis-inducing protein[23]. Results show that Al has likely the ability to damage the genome or to disrupt cellular metabolic processes.

Studies on nipple aspirate fluid (NAF) found significant increased levels of Al in cancer patients and significant correlations of Al with levels of protein oxidative carbonyls, pro-inflammatory IL-6 cytokine and pro-inflammatory monocyte chemoattractant MCP-1 cytokine[104].

These results support that Al ions are involved in oxidative and inflammatory stress of the breast microenvironment, suggesting Al accumulation in breast structures like NAF and tissue as a possible risk factor for oxidative/inflammatory phenotype of breast cells[104].

4.4 Summary

A summary concept of Al salt involvement in breast cancer development is presented by Mannello et al., (2013) and recently in-vitro studies contribute to a more detailed picture of the possible mechanism: After the application of Al-containing UCPs and the absorption of Al through shaved skin, Al-salts may diffuse through epithelial and myoepithelial cells in the extra-cellular matrix, containing leukocytes, fibroblasts and adipocytes. Al ions would be able to alter iron-related metabolism and proteins, releasing Fe^{3+} interacting with Al superoxide-complexes ($AlO_2^{\bullet 2+}$) inducing Fenton reactions and releasing ROS in breast microenvironment. ROS promote an inflammatory

status and long term, repeated Al exposure may lead to a bypass of p53/p21Waf1-mediated cellular senescence[24] and mutations of genes regulating cellular proliferation, migration, metastasis and apoptosis could follow[23,24,67,105]. Ongoing inflammatory status will lead to the release of pro-inflammatory cytokines and chemokines, well known cancer risk factors in human breast microenvironment[104]. (Figure 8)

Although there is experimental in-vitro and in-vivo evidence[22–24,67,104–107] that Al contributes to the development of breast cancer, epidemiological studies show conflicting results[108–110]. Therefore, latest systematic reviews were not able to provide conclusive evidence[111,112].

Mirick et al., (2002) and Fakri, (2006) found no significant associations between antiperspirants and increased risk of breast cancer. In contrast, McGrath, (2003) found that patients using Al containing UCPs frequently received their breast cancer diagnosis at an earlier age than patients avoiding UCPs. However, none of these studies included breast tissue measurements of Al with regard to UCP use. There was so far, no controlled study investigating the relationship of Al with breast cancer combining an epidemiologic approach with breast tissue measurements.

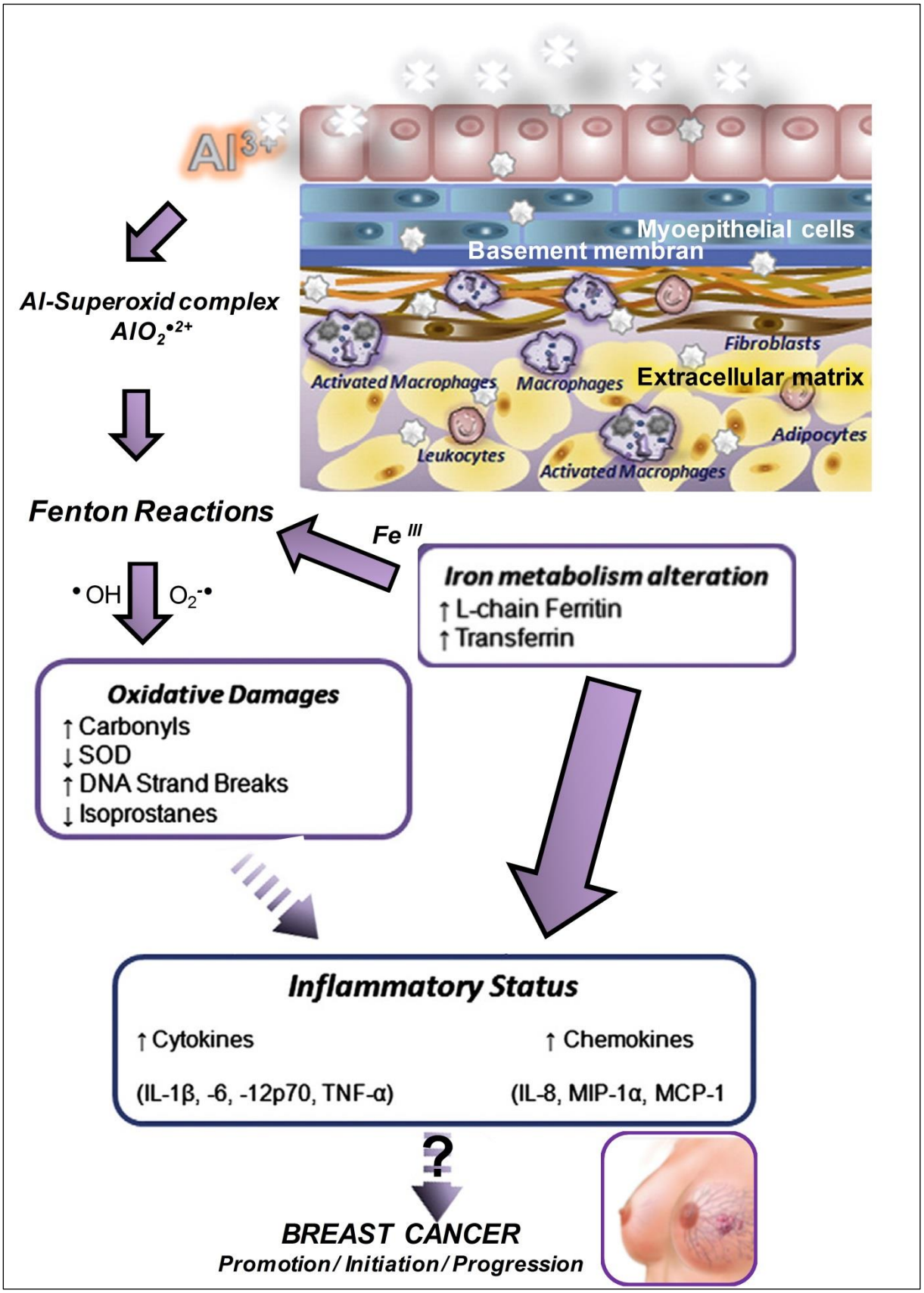

Figure 8 Schematic representation of the involvement of aluminium salts in human breast microenvironment. Modified after Mannello et al. (2013)

5. Own work

In autumn 2011 we started to design and organize the case-control study. I was invited to participate from the very beginning on: during study development, including evaluation of the questionnaire and submission to the ethics commission. Later on I helped to organize to implement the study in the clinic. I settled the sampling protocol for tissue, blood and urine together with clinicians and biochemists and I developed with national and international project partners the protocol for bio-specimen preparation for Al measurement with AAS. I was also involved in the supervision of 5 diploma students and two student co-workers. I was responsible for data and tissue sampling and study coordination, including communication with clinicians, nurses, biochemists, biologists and statisticians, as well as students. I established and managed a data and bio-bank and instructed involved master students. I prepared and digested bio-samples for Al analysis with AAS, contributed to the measurements, supervised by Heribert Talasz and Prof. Herbert Lindner. Finally I analysed the data derived from personal interviews and bio sample analysis and wrote the final manuscript mentored by my supervisor Prof. Hanno Ulmer.

5.1 Summary of published results: UCP use, Al levels and BC (Full paper)

Use of Underarm Cosmetic Products in Relation to Breast Cancer: A Case-Control Study.

5.1.1 Background

Breast cancer is the most common cancer in women with a high prevalence in economically developed countries[113,114] and belongs due to its multifactorial aetiology to the so called NCDs. Age, genetic mutations and life-time oestrogen exposure are well known risk factors[115–117] but explain only a small part of the aetiology[118] suggesting that environmental factors contribute to breast cancer development[119,120]. The frequency of tumour occurrence in the upper outer part of the breast has increased in recent decades[71,73,75,76] pointing to a possible role of underarm cosmetic products in the aetiology of breast cancer[73,75–77]. In parallel, results of preclinical work showed possible carcinogenic effects of Al salts which are the active ingredients of underarm cosmetic products. Three studies investigated the effect of underarm cosmetic products on breast cancer in humans with conflicting results. None of these studies measured Al in tissue.

We conducted a 1:1 age-matched hospital-based case-control study aiming to investigate the risk for breast cancer in relation to self-reported UCP use. We included Al measurements in breast tissue from a subgroup of breast cancer patients and healthy individuals. We hypothesized that (1) breast cancer patients had used UCPs more frequently during their lives than healthy controls, that (2) Al concentrations in breast tissue are increased in cases, and that (3) there is a relationship between UCP use and measured Al concentrations in breast tissue.

5.1.2 Methods and results

In summary, 2010 women with breast cancer and 250 healthy women without malignant BC history were interviewed between January 2013 and October 2016 on BC risk factors and life style, including questions about hygiene habits, UCP use, shaving, nutrition, alcohol consumption and physical activity. Eligible cases were BC patients aged 20-85 who had confirmed diagnosis of BC within the last 5 years. Interviews were conducted at the Department of Obstetrics and Gynaecology, at the Department of

Plastic, Reconstructive and Aesthetic Surgery or at other departments of the Medical University of Innsbruck, Austria. The questionnaire used in these interviews was a modified version of the validated questionnaire used in the MARIE study[121], extended by specific questions regarding personal hygiene, UCP use and Al exposure. Questions asked refer to past exposure in four lifetime categories: *'under the age of 30 years'*, *'between 30 and 50 years'*, *'over the age of 50 years'* and *'last five years before breast cancer diagnoses'*.

Most women were not able to distinguish between deodorants and antiperspirants. We therefore concluded to summarize them with 'underarm cosmetic products' (UCP) as the main exposure variable. UCP application categorized in *'never', '1–4 times per month', '2–6 times per week', 'daily'* and *'several times per day'* was defined as the primary endpoint of this study.

Each BC patient was age-matched in a 1:1 ratio to one healthy woman, minimizing the age difference within case-control pairs by a validated matching algorithm resulting in 209 age-matched pairs. The application of the SPSS algorithm ensured an objective and random assignment of cases to controls in order to reach the optimum result in terms of age difference.

Additionally breast tissue samples à 500 mg were sampled from 100 cases and 52 controls near axilla, near mammillae and near sternum. Sampling was done on mastectomy preparations respectively during breast reduction surgery (controls). In cases, we took samples of the breast affected by the tumour, in controls sampling was performed on both breasts. (Figure 10A)

Prior to Al analysis, tissue samples had to be defatted and digested in order to analyse a clear fluid with graphite furnace atomic absorption spectrometer (GF-AAS) as described in Exley et al., (2007). Thawed tissue was defatted by incubation at 37 °C for maximal 72 hours to assure constant weight. Further tissue preparation, digestion and dilution were done according to House et al., (2013). For digestion high quality Nitric acid 69% Trace SE- LECT® (Sigma-Aldrich, Germany) was used. Digested and diluted tissue samples as well as ninety method blanks were analysed then as clear fluids with GF-AAS using Zeeman-effect background corrector (Thermo Scientific, Germany).

Self-reported history of UCP use was compared between 209 female BC patients (cases) and 209 healthy controls with an multivariable conditional logistic regression analysis to estimate odds ratios (ORs) with 95% confidence intervals (CIs), adjusting for established BC risk factors. Al concentrations were averaged per women, summarized

with medians and interquartile ranges (IQR) for cases and controls and stratified by UCP application. Summarized Al concentrations were compared between cases and controls with an independent t-test. A three-way ANOVA for repeated measurements with the between-subject factor *'case versus control', 'UCP use'* as ordinal scaled covariate, and the within-subject factor *'sampling location'* were performed on log10(x+1) Al concentrations. We performed subgroup analysis for Al measurements separately for cases with tumours in the upper outer quadrant and tumours in other quadrants.

Crude ORs from univariable regression analysis confirmed established breast cancer risks. As expected positive family history of breast cancer was the most pronounced risk factor. Further characteristics that were significantly different between cases and controls were a family history of other cancers such as prostate, ovarian and endometrium cancer and history of benign breast disease. (Table 4)

Table 4 Matching variable 'Age' and significant self-reported characteristics of breast cancer patients and healthy controls.

Risk factor	Cases (n=209)	Controls (n=209)	Crude OR (95% CI)†	p-value
Age at interview [years, means (SD)]	51·9 (12·0)	51·8 (12·1)		0·2994
Family history of breast cancer (%)	76 (36·4)	32 (15·3)	2·91 (1·81-4·68)	<0·0001
Family history of other cancer (%)	128 (61·5)	103 (49·3)	1·60 (1·09-2·35)	0·0176
History of benign breast disease (%)	63 (30·1)	43 (20·6)	1·61 (1·04-2·48)	0·0326

†derived from univariable conditional logistic regression analysis.

Use of UCP was significantly associated with risk of BC (p=0.036). The risk for BC increased by an OR of 3.88 (95% CI 1.03–14.66) in women who reported using UCPs several times daily starting at an age earlier than 30 years. However if not significant for every subcategory, crude and adjusted OR of 'UCP use under the age of 30' and 'UCP use during the last 5 years before BC diagnosis/interview' increased with the amount of UCP application. (Table 5)

Table 5 Use of underarm cosmetic products (UCP) in women with breast cancer and healthy controls

	Number of cases (%) (n=209)	Number of controls (%) (n=209)	Crude OR (95% CI)	Crude p-value	Adjusted OR† (95% CI)	Adjusted p-value
UCP use under the age of 30				0·0951		0·0358
Never	43 (20·6)	46 (22·0)	reference		reference	
1-4 times per month	19 (9·1)	26 (12·4)	0·83 (0·40-1·73)	0·6222	0·50 (0·20-1·26)	0·1435
2-6 times per week	26 (12·7)	36 (17·2)	0·87 (0·43-1·75)	0·6930	0·53 (0·23-1·25)	0·1486
Daily	103 (49·3)	89 (42·6)	1·40 (0·79-2·53)	0·2603	1·03 (0·51-2·07)	0·9390
Several times per day	18 (8·6)	9 (4·3)	2·84 (1·02-7·89)	0·0451	3·88 (1·03-14·66)	0·0456
Unknown	0 (0·0)	3 (1·4)				
UCP use during last 5 years*				0·1104		0·0822
Never	25 (12·0)	34 (16·3)	reference		reference	
1-4 times per month	24 (11·5)	21 (10·0)	1·67 (0·73-3·81)	0·2211	1·41 (0·49-4·04)	0·5216
2-6 times per week	31 (14·8)	45 (21·5)	0·99 (0·49-2·02)	0·9824	0·59 (0·25-1·40)	0·2338
Daily	109 (52·2)	96 (45·9)	1·70 (0·90-3·21)	0·1046	1·22 (0·56-2·66)	0·6105
Several times per day	20 (9·6)	13 (6·2)	2·63 (1·00-6·87)	0·0492	3·16 (0·90-11·15)	0·0736
Unknown	0 (0·0)	0 (0·0)				

†Adjusted for age at interview, age at menarche, parity, age at first live birth, menopausal status, age at menopause, MHT drug therapy, history of breast cancer, history of benign breast disease, family history of other cancer, BMI, alcohol consumption in multivariable conditional logistic regression analysis.

*In cases: UCP use during the last 5 years before BC diagnosis respectively in controls during last 5 years before the interview.

Al levels in tissue ranged from 0.00-367.28 nmol/g, with highest values for women with a tumour in the upper outer quadrant. Al levels in tissue were zero inflated and skewed and therefore log10(Al+1) transformed. Cases showed more outliers of high Al concentrations (Figure 9B) and measured Al were significantly higher (independent t-test, $t_{149.8}$=-3.25, p= 0.001) in cases than in controls (5.8, 2.3–12.9 versus 3.8, 2.5–5.8 nmol/g, p=0.0014, Figure 9C and D).

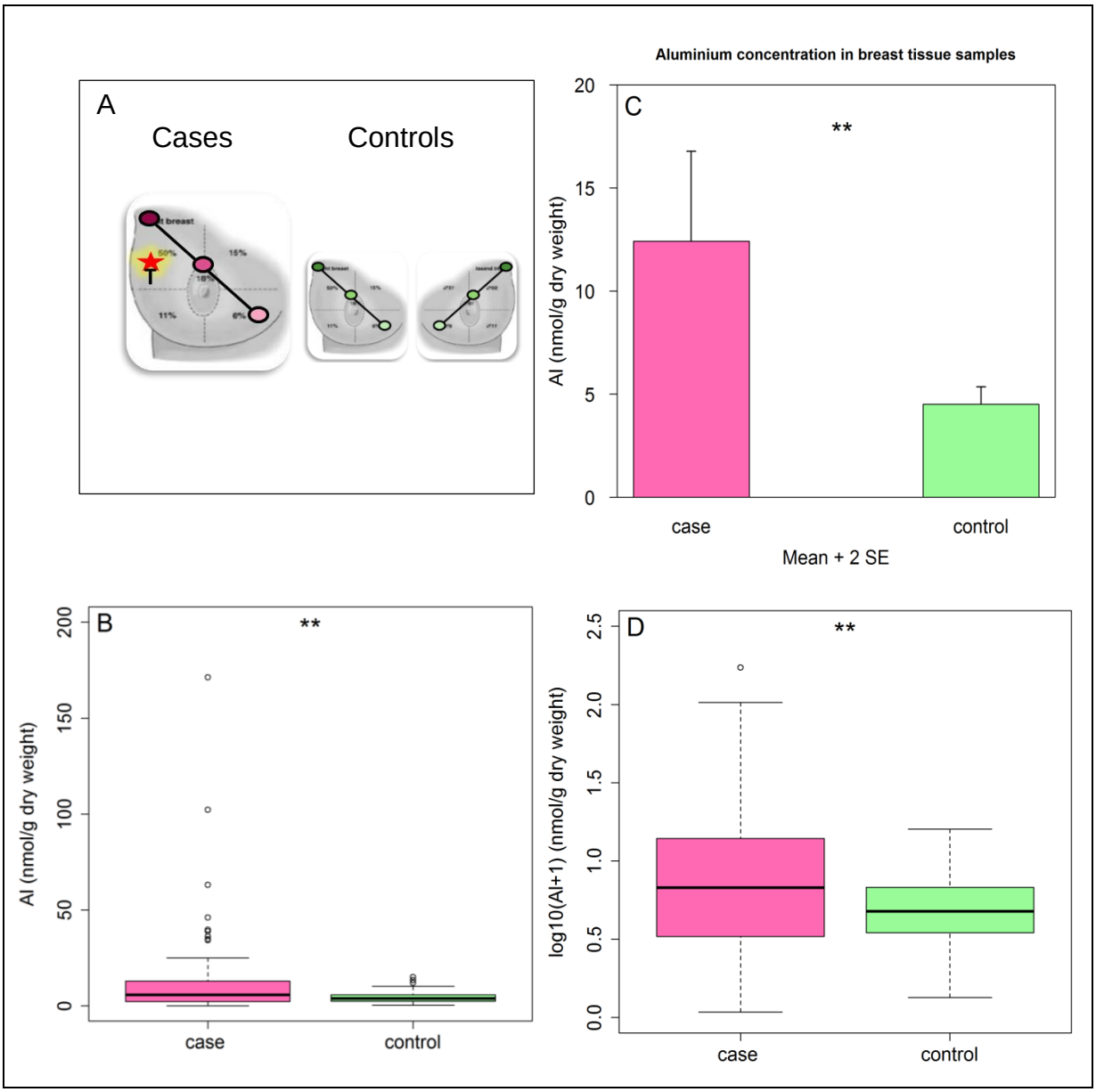

Figure 9 A) Sampling scheme of the three tissue sampling sites per breast in women with BC (pink) and healthy controls (green). B) Aluminium levels of 3 sampling sites per case and 6 sampling sites per control were averaged per woman. C) Untransformed and B) transformed Al levels (nmol/g dry weight) in breast tissue samples of cases and controls. In B, C, D: significant differences between Al levels in tissue of cases and controls (p=0.0014) indicated with asterisks (**).

The factor *'UCP use under the age of 30'* was significant related to Al levels in tissue (two-way ANOVA, $F_{1,147}$=4.56, p=0.034) as well as *'UCP use during the last 5 years before BC*

diagnosis respectively before interview' (two-way ANOVA, $F_{1,147}$=6.96, p=0.0093). The results stratified for tumours localization showed significant differences in Al levels between cases and controls in the subgroup of cases with a tumour in the upper outer quadrant only (independent t-test, $t_{80.1}$=-3.75, p<0.001). Also a significant relation to Al levels and UCP use was observed in the subgroup of cases with tumour in the upper outer quadrant only (*'UCP use under the age of 30'*: three-way ANOVA, $F_{1,103}$=6.61, p=0.0116 *and 'UCP use during the last 5 years before BC diagnosis respectively before interview'*: three-way ANOVA, $F_{1,103}$=7.34, p=0.0079, Figure 10A)

Al levels of cases with an tumour in lower or inner quadrant were not significant higher than in controls (independent t-test, $t_{63.9}$=-1.13, p=0.264) and were not related to UCP use *('UCP use under the age of 30'*: three-way ANOVA, $F_{1,93}$=0.90, p=0.0356 and '*UCP use during the last 5 years before BC diagnosis respectively before interview'*: three-way ANOVA, $F_{1,93}$=2.31, p=0.373, Figure 10B)

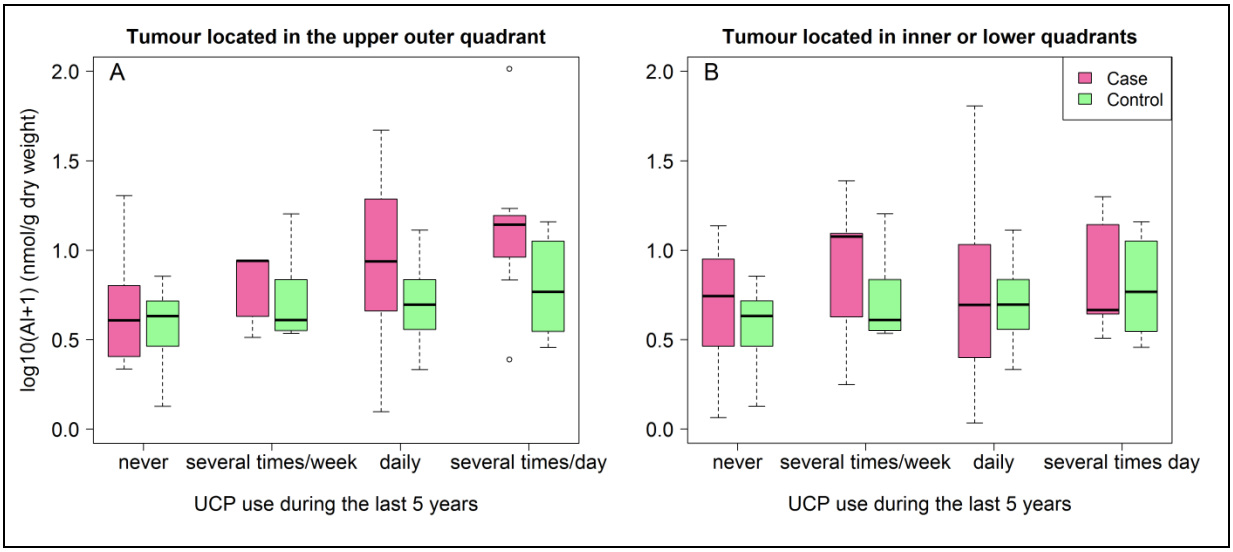

Figure 10 Median and (IQR) range of Al concentrations related to UCP use in the last 5 years before BC diagnosis in cases, and 5 years before the interview took place in controls. Cases with an tumour located in the upper outer quadrant (A) showed a significant relation of Al concentration and UCP use (p=0.008), but cases with an tumour in other quadrants (B) did not (p=0.132).

5.1.3 Discussion

The findings suggest an association between UCP use, Al concentration in breast tissue and breast cancer. With increasing UCP use ORs of UCP application categories rises from never to several times per day, suggesting a dose-response relationship. However, the significant association of UCP use and breast cancer was limited to women who reported using UCP's several times a day when they were under the age of 30.

Previous epidemiologic studies[108–110] did not support the hypothesis that UCP use is associated with the risk for breast cancer. Reasons might be a small sample size,

underpowered to detect realistic effect sizes[110] or an unmatched and uncontrolled study design[108,110]. Regarding to our results also the dichotomous categorisation of UCP-use as evaluated by Fakri, (2006) and Mirick et al., (2002) are too coarse to detect an effect, when a significant association was only observed by intense use like several times a day. Also data about UCP use during different life times were not included by previous studies and therefore possible effects of UCP use at sensible younger ages, like during puberty, were not detectable.

The birth cohorts of breast cancer patients recruited in the study of Mirick et al., (2002) were diagnosed in the early 1990ties, on average 20 years earlier than patients in our study. UCP use strongly increased in the last four decades and also cultural habits such as shaving of axilla hair became only popular during the late 1980ties in western countries[73,76,108] It is likely that participants in the study of Mirick et al., (2002) were less exposed to Al by UCP use when they were under the age of 30 (approximately in the 1940ties and 1960ties), than women 20 years later who participated in the Innsbruck Al-breast cancer study.

Regarding, the analytical approach the measured Al concentrations in our cohort were similar to those in former studies[79,123]. None of the previous studies sampled control tissue from healthy individuals. We observed a significant difference regarding Al concentrations of cases and controls and a significant association between Al concentration in tissue and UCP use, suggesting dermal Al absorption. Furthermore after subgroup analysis for tumour localisation, differences in Al concentrations between cases and controls and Al as well as the association between Al and UCP use were only evident when restricting analyses to cases with tumours in the upper outer quadrant, supporting the hypothesis of previous studies[73,75] that Al in UCPs contributes to tumour development in upper outer quadrants. But results of the questionnaire part of our study do not support this hypothesis. Self-reported UCP use did not differ between cases and controls when considering tumour localization.

Al concentration of cases showed a higher variation than in controls, and on certain spots Al concentration in tissue of cases was even higher than 100 nmol/g wet weight. The Al levels in tissue of ten patients were in the range of Al concentrations transforming cultured mammary epithelial cells in-vitro and enabling them to form tumours and metastasis in mouse models[23].

Our study is though the combination of questionnaire data and biosample analysis of cases and controls to date the most comprehensive study concerning Al, UCP use and

breast cancer, although there are several limitations. First, data was sampled retrospective, a case-control study is susceptible to recall bias. Second, the mix of incident and prevalent cases may be an additional source of bias. Although there is no significant effect modification of the different timing of interviews (p=0.282, for the 'UCP use under the age of 30'-model, p=0.877 for the 'UCP use in the last 5 years'-model), we cannot rule out any recall issues between incident and prevalent cases.

Reporting bias cannot be excluded; we tried however to reduce it by performing interviews with well-trained interviewers and study participants were blinded as to the study purpose, avoiding the overfocusing on Al and UCP use.

Compared to previous studies[108,109] the sample size of the study was smaller and the limited sample size of the study leads to relative small numbers in the sub categories of the main exposure variable.

Reverse causation effects regarding significant differences in Al concentration of cases and controls can not be excluded, meaning that the breast tumour may accummulate Al like other transition metals[124–126]. Although, we matched cases and controls on age, the subgroup for tissue sampling is not age matched. However, in our study, Al concentrations did not correlate with age (r=−0.028, p=0.7291, n=99).

However UCP use was only significant for the highest application category *'several times per day'* and other application categories did not differ between cases and controls especially if UCP use is measured in a dichotomous way only. Self-reported UCP use did not differ between cases and controls when considering tumour localization. However Al concentration was significant higher in cases with tumour localization in the upper outer quadrant and UCP use was significant related to Al concentration. These incoherences between the results of the questionnaire part and the tissue part while showing quite the same amount of exposure but different incorporated Al concentrations might point to the effect of Al excretion. It is possible that some women are more effective in Al excretion through sweating or urinary Al excretion.

5.1.4 Conclusion

This hospital-based case-control study provides novel insights and additional evidence regarding a possible role of UCP use and Al salts in the aetiology of BC. We observed an increased risk for BC in women who reported more than daily use of UCP when they were under the age of 30 and that the frequent use of underarm cosmetic products led to an accumulation of Al in breast tissue. Our findings suggest an association of Al levels

in tissue and UCP use obvious for BC patients with a tumour in upper outer quadrant, suggesting a contribution of Al to tumour development in upper outer quadrant.

We observed high SDs (max=170.38) and wide concentration ranges (min=0.16, max=308.4,) between the three sampling sites taken from a woman's breast. These findings support the thesis of a patchy and unequal Al distribution in breast tissue[79,122] and lead to the assumption that Al probably concentrates on certain spots up to mutagenic levels as reported in the most recent and comprehensive in-vitro study[23].

Until definitive answers about the involvement of Al in the process leading to breast cancer, we recommend that women at their younger ages should be careful with the use of UCPs and avoid their excessive use.

5.2 Summary of unpublished results

In the following section unpublished results concerning age of first diagnosis in relation to UCP application and other self-reported data from the questionnaire together with Al concentration in bio-samples (urine, blood and tissue) are presented.

5.2.1 UCP use and mean age of breast cancer diagnosis

Background

The study of McGrath, one of the two epidemiologic studies about BC and UCP use, showed that frequent UCP application is related to an earlier age of BC diagnosis. Women who tend to use very often UCPs received their diagnosis at a significant earlier age[108]. The findings of McGrath were compared with data of BC patients participating in the Innsbruck study on BC and Al. To analyse the possible correlation of UCP use and earlier age of BC diagnosis a one-way ANOVA with Bonferroni corrected post-hoc test for subcategories of UCP use was performed.

Results

More frequent use of UCP application was significantly related to an earlier BC diagnosis ($F_{4,209}$=20.2, p<0.001). Women who never used an UCP received significantly later their BC diagnosis than women who used it *2-6 times per week*, *daily* or *several times per day* a (p<0.001). Also women who used UCP's just *1-4 times per month* were significant older when diagnosed with BC than women who used UCPs *daily* (p=0.007) or *several times per day* (p=0.003). (Figure 11)

Discussion

The results support the hypothesis and findings of McGrath, (2003) that an earlier age of BC diagnosis is related to more frequent use of UCPs. But there are differences between the Innsbruck study and McGrath's study concerning the mean age of UCP application categories. McGrath, (2003) reported a mean age of BC diagnosis for women with a maximal use of UCPs, defined as an application of 2-5 times per week up to several times per day, of 52.6 years. Women with a maximal UCP use (several times per day) participating at the Innsbruck study received their diagnosis with a mean age of 42.1 years, already 10 years earlier. Reasons might be minor methodical aberrations and differences in the mean age of the recruited patient cohort. In contrast to the Innsbruck

study, McGrath, (2003) used four usage groups instead of five and included the life-style habit of underarm shaving together with UCP use in the usage groups.

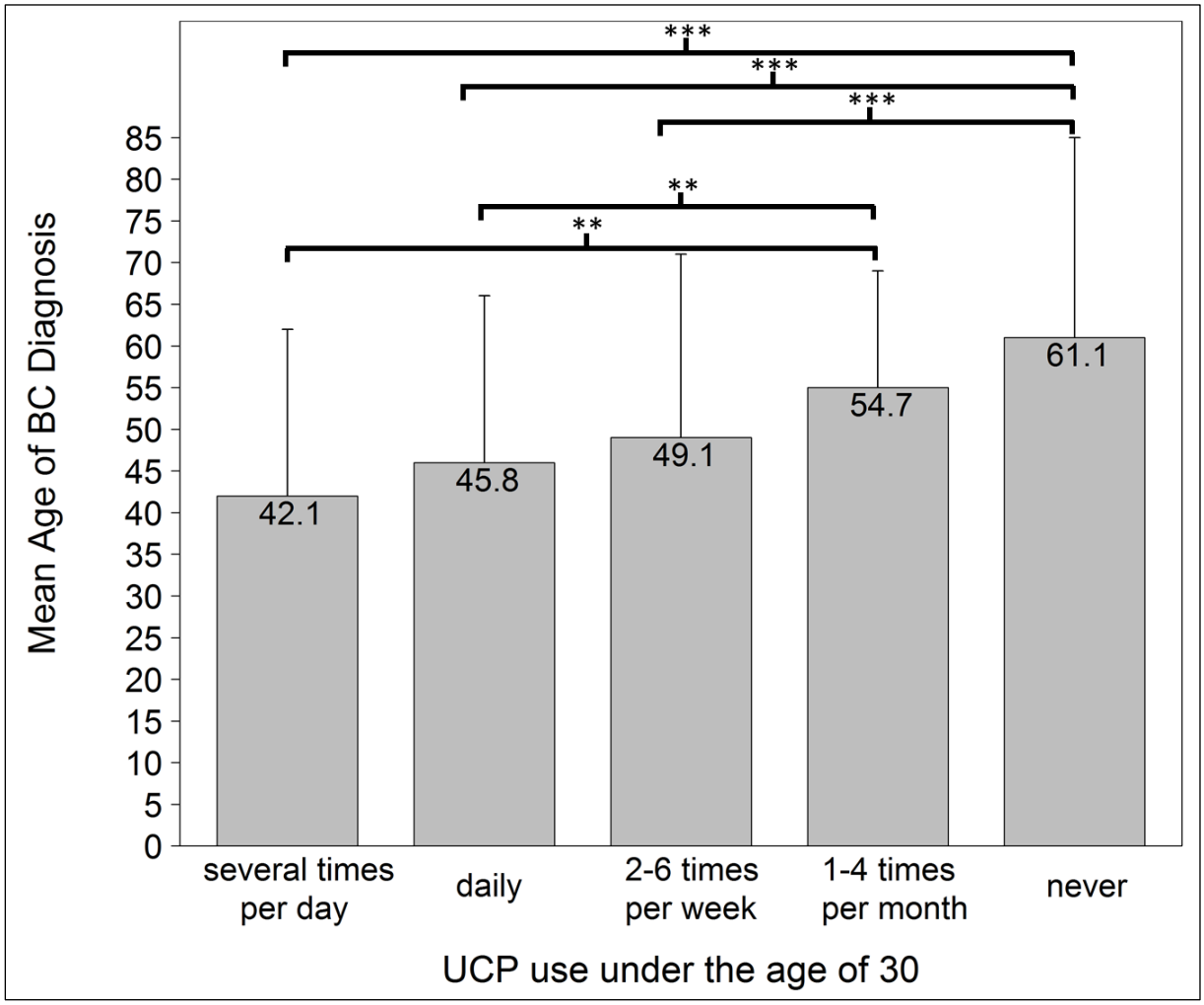

Figure 11 UCP use under the age of 30 for 5 different usage groups and mean age of breast cancer diagnosis. A higher UCP use leads to a significant earlier BC diagnosis. (N=209, $n_{several\ times\ per\ day}$=18, n_{daily}=103, $n_{2\text{-}6\ times\ per\ week}$=26, $n_{1\text{-}4\ times\ per\ month}$=19 and n_{never}=43 women)

The mean age of the recruited patient cohort of the Innsbruck study was 51 (±12) whereas the mean age of participants in McGraths study was 66 (±13).

The difference in mean age of BC diagnosis between the study populations may occur due different diagnosis methods applied over the last decades. Women participating in McGraths study were diagnosed with BC between 1993 and 2002, whereas women participating in the Innsbruck study received their BC diagnosis between 2008 and 2017. Also differences between US and Austrian health care programmes like mammography screenings might be a reason for the discrepancy of age differences between the study cohorts.

Although the results support the findings of McGrath, (2003), the relation of earlier BC diagnosis with more frequent use of UCPs might be biased due to changes in life-style

habits over time and retrospective uncontrolled studies are likely affected by age-dependent reporting bias.

5.2.2 Al levels in blood and urine of BC patients and healthy controls

Background

It is assumed that urinary excretion is a major route for systemic Al excretion, with up to 100 µg of excreted Al per day[30]. Al is mainly transported in blood via serum transferrin but whole blood levels showed significant higher Al levels than serum[127] and reported levels[128] ranged from 0 µg/L up to 100 µg/L. Reported urine levels[128,129] were about 5.4-9 µg/L. The mean concentration of Al in a non-exposed population, who did not use antacid drugs, was 0.06 µmol/L (SD=0.03, range 0.02-0.13, n = 21) in serum, and 0.33 µmol/L (SD=0.18, range 0.07-0.82, n=44) in urine. The upper reference limit for Al in a healthy, non-exposed population was estimated to be 0.1 µmol/L in serum and 0.6 µmol/L in urine[130].

Al levels in blood and urine of BC patients and healthy controls participating in the Innsbruck study were compared to literature data of a non-exposed population.

Furthermore Al levels of blood and urine were compared between healthy individuals and BC patients and physiological differences of Al accumulation (blood) and excretion (urine) were investigated. Together with Al levels in tissue, the levels in blood and urine should help to get a more comprehensive picture of the Al body burden.

Methods

Urine was sampled during hospitalisation, on the day before surgery, in pristine, acid-washed polyethylene urine containers (Sarstedt, UriSet 24). Urine samples were carefully collected by patients under supervision of nurses, who were instructed to help reduce issues related to potential contamination[20]. Urine of each patient was sampled for at least 6 hours. After collection the containers were thoroughly mixed and one urine subsample per patient was collected with a 10 ml Sarstedt-Monovette®. 5 ml blood samples were taken during routine venepuncture (Sarstedt-Monovette®, 4.9 ml, Lithium-Heparin Gel liquid). Blood and urine samples were stored at the Lab of Clinical Biochemistry, Gynaecology Department, at -20°C until analysis at the Division of Clinical Biochemistry, CCB, Medical University Innsbruck. Blood and urine samples were defrosted, carefully vortexed and charged with hydrogen peroxide and nitric acid respectively. Prior to analysis with GF-AAS, samples underwent a microwave digestion.

Additional 30-40 method blanks were performed. Measured background values of method blanks were subtracted from measured Al levels in blood and urine samples. Urine levels were standardized to creatinine levels of each patient, measured by the Central Laboratory, Medical University Innsbruck (ZIMCL).

Values of Al levels in urine and blood were highly zero inflated and skewed. Therefore blood and urine values were log10(x+1) transformed and analysed with an unpaired t-test to compare cases and controls. A Spearman rank-order correlation was conducted in order to determine a relationship between Al levels in blood and urine.

Results

Al in tissue was significant positive related to Al in blood (Spearman's r=0.3, n=99, p=0.01). Subgroup analysis showed also a significant, positive correlation for Al levels in blood and tissue of cases (Spearman's r=0.3, n=68, p=0.028) but not for controls (Spearman's r=0.2, n=31, p=0.314).

Al amount in urine was not related to Al in tissue (Spearman's r=0.05, n=107, p>0.05). There was also no significant correlation of Al in urine and blood (Spearman's r=0.2, n=107, p=0.083).

Median and IQR of Al levels in blood of cases were 0.3 (0.0-3.8) µg/L and of controls 0.0 (0.0-2.0) µg/L, respectively, but did not differ significantly (t_{107}=-1.44, p=0.110). Median (IQR) Al levels in urine of cases and controls were 1.6 (0.0-5.5) µg/g Crt respectively 1.5 (0.0-5.2) µg/g Crt in controls and did not differ significantly (t_{105}=0.38, p=0.970).

Subgroup analysis for tumour location of cases with tumours in the upper outer quadrant compared to controls showed neither significant different Al levels in blood samples (p=0.066) nor urine samples (p=0.308). Also subgroup analysis for cases with tumours in other quadrants compared to controls did not show significant differences in Al levels of blood and urine samples (urine: p=0.711, blood: p=0.739).

Mean (SD) of Al in blood of cases with a tumour in the upper outer quadrant was 7.3 (17.3) µg/L, of cases with a tumour in other quadrants 2.4 (4.4) µg/L and measured Al in blood of controls was 2.0 (3.7) µg/L.

Mean (SD) of Al in urine of cases with a tumour in the upper outer quadrant was 4.7 (8.9) µg/g Crt, of cases with a tumour in other quadrants 3.5 (6.4) µg/g Crt and measured Al in urine of controls was 3.0 (3.5) µg/g Crt (Figure 12).

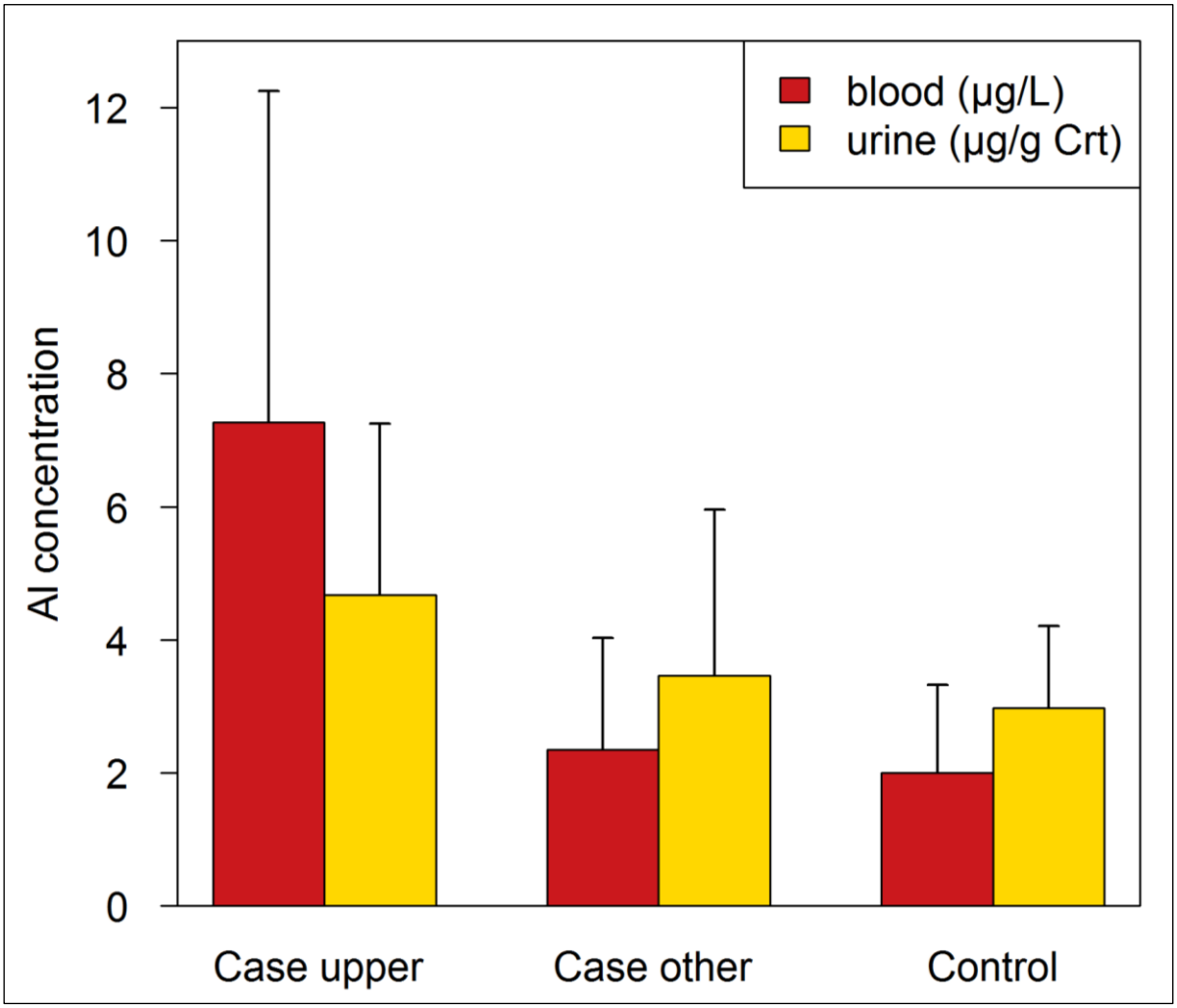

Figure 12 Mean Aluminium concentration and SD in blood and urine samples of cases (n=76) with a tumour localisation in the upper outer quadrant (Case upper, n=48), for cases with tumours in inner or lower quadrants (Case other, n=28) and for controls (n=32).

Discussion

Measured Al levels in blood and urine of cases and controls were similar to the Al levels measured in unexposed humans[127,128,130]. Differences in blood and urine levels between cases and controls were not significant, although cases showed higher mean Al levels in blood and urine (Figure 12). Cases with a tumour in lower or inner quadrants showed same blood/urine proportion of Al whereas cases with a tumour in the upper outer quadrant showed an inverse proportion of Al µg/L in blood to Al µg/g Crt in urine. (Figure 12). This non-significant observation led to the assumption that some women suffering from BC may excrete Al less efficient than others.

There was a tendency of higher Al levels in blood and urine of cases compared to controls, especially in samples of cases with a tumour in the upper outer quadrant. But due to a low sample size (76 cases, 32 controls) this tendency was not confirmed. One urine sample per patient is likely a snap-ready method without reliable information. To

investigate the body burden of Al and it's excretion by urine, it would be necessary to collect urine samples over a longer time, at least for 24 hours or over several days. Al in blood and tissue samples correlated significantly while there was no correlation with Al in urine samples, leading to the question if other excretion mechanisms like sweat are more efficient[131].

5.2.3 Possible gradient of Al from axilla to sternum

Background

A change in the topological distribution of mammary carcinoma since 1975[71–73,75,76] towards a higher incidence in the upper outer quadrant seems to point to UCPs as a potential contributor assuming higher incorporated aluminium near the axilla than near mammillae or sternum[67,73,74,76]. The publication by Exley et al. (2007) including 17 participants detected significantly higher Al levels in the two outer breast regions (axilla + lateral) compared to the two inner breast regions (middle + median)[122]. For the Innsbruck study on BC and Al three tissue samples alongside the transect axilla—mammillae—sternum were sampled from mastectomy preparations of BC patients and from removed breast tissue of women undergoing breast reduction surgery (Figure 9A). To assess a possible gradient in Al concentration from axilla to sternum a Jonckheere-Terpstra trend test was performed on medians of each sampling location.

Results

There was a decrease in Al concentration from the sampling site near axilla to the sampling site near the sternum, in the lower inner quadrant (Figure 13) But this gradient of Al was not significant, neither for all study participants (p=0.445) nor for cases (p=0.699) or controls (p=0.451) and also not for women with a tumour in the upper outer quadrant (p=0.533) or for women with tumours in other quadrants (p=0.940,).

Table 6 Mean (SD) of Al levels (nmol/ g dry weight) in the sampling sites from axilla to sternum and results of Jonckheere-Terpstra trend test.

	axilla	mammillae	sternum	p-value	n†
All women	11.24 (33.06)	9.61 (17.52)	7.73 (12.25)	0.445	152
Case	14.48 (40.31)	12.25 (20.98)	9.75 (14.61)	0.451	100
Control	5.00 (4.62)	4.53 (3.81)	3.89 (3.89)	0.699	52

†Jonckheere-Terpstra trend test on medians of each sampling location.

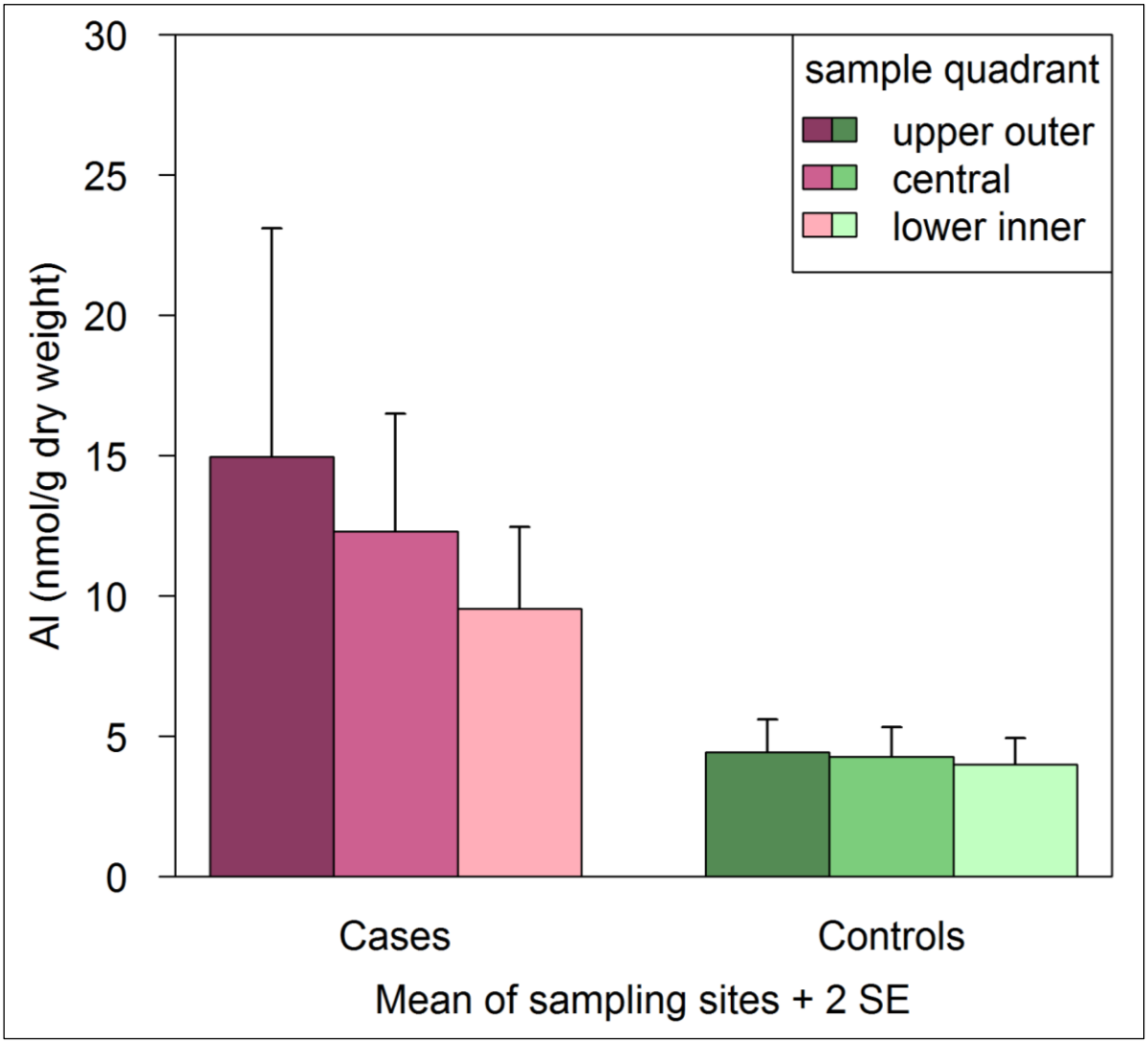

Figure 13 Mean (2*SE) of Al levels in tissue samples from the tumour breast and the right breast of controls: From axilla, the nearest sampling side of Al exposure by UCP application, to sampling site near mammillae and sampling site near sternum, with highest distance to UCP application. (cases: n=100, controls: n=52)

Discussion

Highest Al levels were measured near the axilla and near the mammillae and decline near the sternum, but without significance for all participants and subgroups. SDs are in general very high pointing to a very unequal distribution of Al in tissue. To investigate Al distribution and concentration on certain spots more detailed data about individual breast morphology (breast density, distribution of lymph and blood vessels) would be helpful. For quadrant comparisons and to identify a significant gradient from axilla to sternum at least two samples per quadrant, like in the protocol of Exley et al (2007), are necessary in order to avoid an under-powered study design for this research question[122].

5.2.4 UCP application after shaving and Al levels in tissue and blood

Background

The shaving of armpit hair is a usual cultural practise, especially in western countries. For many women it is common to shave armpit hair prior to UCP application, negating the specific warning by the FDA and EU[132] although shaving could create cuts which may provide easy routes for cosmetic ingredients[74,133]. The study of McGrath (2003) showed that an earlier start of antiperspirant/ deodorant usage and a more frequent use together with underarm shaving were associated with breast cancer diagnosis at younger age[108]. It is assumed that the application of UCPs after shaving is related to higher Al absorption by impaired stratum corneum via cuts and micro lesions[134] and is likely the main source of Al for the underarm dermis, underlying tissues and the mammary epithelium[23]. There are studies on Al absorption through mouse[87] and human skin[68,69]. One in-vitro study[68], including a man and a women as participants, demonstrated unequivocal Al absorption through intact skin and its excretion in urine. However the Al uptake was small but the long-term effect of low dose uptake or uptake via shaved skin was not considered. In an in-vitro experiment shaved skin, modelled by stripped skin, was more permeable for Al than intact skin[69]. Comprehensive in-vivo studies on Al levels in tissue and blood in relation to data on Al exposure via UCP use and shaving are lacking, therefore we collected these data in the course of the Innsbruck study on BC and Al.

The results of the questionnaire regarding UCP application after shaving were compared to Al levels in bio-samples of tissue and blood of cases and controls. Study participants were asked whether they applied during the last 5 years a UCP directly after they had shaved or removed their underarm hair or whether they separated these hygiene habits respectively if they did not shave or apply a UCP. These questions were summarized in the categories shaving and UCP application *'separated'* or *'together'*. To examine the influence of the life style habit *'UCP application after shaving'* Al levels in **blood** and **tissue** were log10(x+1) transformed and analysed by an independent t-test. Bonferroni-correction was used to correct for multiple testing between subgroups.

Results

Univariate logistic regression analysis showed an increased OR (OR=1.3, 95% CI=0.8-1.9,) for the self-reported life-style habit '*UCP application after shaving'*, but without significance (p=0.265, Table 7).

Table 7 Results of the univariate and multivariate logistic regression analysis regarding 'UCP application after shaving' from 209 matched cases and controls.

Risk factor	Number of cases (%) (n=209)	Number of controls (%) (n=209)	Crude OR (95% CI)†	Crude p-value	Adjusted OR (95% CI)†	Adjusted p-value†
UCP application* after shaving	81 (38.8)	70 (33.5)	1.3 (0.8-1.9)	0.265	1.4 (0.9-2.4)	0.172
No UCP application* after shaving	128 (61.2)	139 (66.5)				

†Adjusted for age at interview, age at menarche, parity, age at first live birth, menopausal status, age at menopause, MHT drug therapy, history of breast cancer, history of benign breast disease, family history of other cancer, BMI, alcohol consumption in multivariable conditional logistic regression analysis.
*In cases: UCP use during the last 5 years before BC diagnosis respectively in controls during last 5 years before the interview

Al in **tissue** related to UCP use after shaving

Women who applied a UCP directly after shaving of axilla hair had significantly higher Al levels in breast **tissue** than women who separated these hygiene habits (t_{149}=-2.2, p=0.032, Table 8, Figure 14A). Women who applied a UCP after shaving had higher Al levels (Table 8), but subgroup analysis showed no significant effect, neither for controls (t_{50}=-1.9, p=0.069) nor for cases (t_{97}=-1.6, p=0.107).

Table 8: Median (IQR) of Al (nmol/g dry weight) in tissue samples of cases and controls stratified by the habit of UCP application after shaving or not.

Al levels in tissue	shaving & UCP separated	N	shaving & UCP together	N	**p-value**† shaving & UCP
All participants	4.1 (2.3-7.7)	88	5.6 (2.4-12.9)	63	0.032
Control	3.3 (1.9-5.5)	31	4.5 (3.0-5.9)	21	0.069
Case	5.3 (2.5-10.9)	57	8.1 (2.0-18.9)	42	0.107

†Independent samples t-test with log10(x+1) transformed data.

Al in **blood** related to UCP use after shaving

Women who applied a UCP directly after shaving of axilla hair had significantly higher Al levels in **blood** than women who separated these hygiene habits (t_{106}=-2.2, p=0.034). The effect of UCP application after shaving was significant for cases ($t_{55.6}$=-2.3, p=0.026) but not for controls (t_{30}=-0.2, p=0.848)

Subgroup analysis revealed a significant effect of UCP application after shaving on Al concentration in blood of women with a tumour in the upper outer quadrant ($t_{44.7}$=-2.8, p=0.008). Women with a tumour in other quadrants did not show significant higher Al levels in blood related to their self-reported UCP application after shaving (t_{26}=0.4, p=0.726, Table 9, Figure 14B).

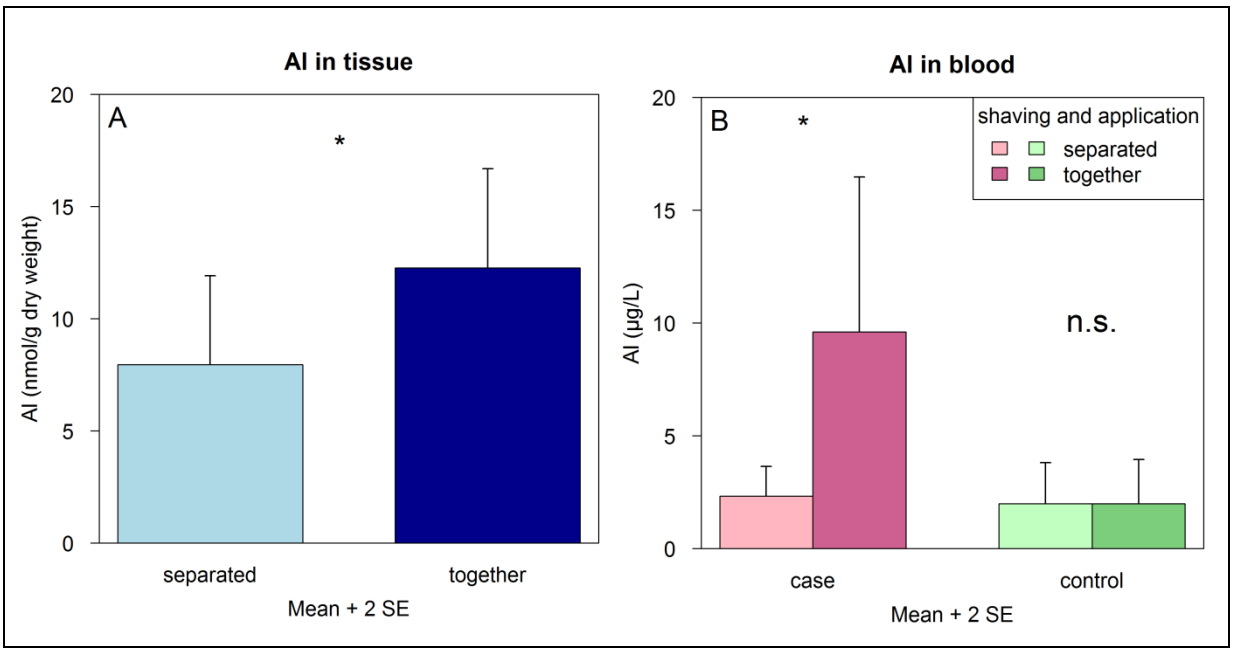

Figure 14 A) Al concentration (nmol/g dry weight) in tissue of women who applied a UCP directly after shaving (together) or not (separated). B) Al concentration (µg/L) of women who applied a UCP after shaving or not in blood of cases and controls.

Table 9: Median (IQR) of Al in blood (µg/L) of cases and controls that applied UCP after shaving or not.

Al levels in blood	UCP & shaving separated	n	UCP use after shaving	n	**p-value†**
All participants	0.0 (0.0-10.9)	61	0.7 (0.0-7.5)	47	0.034
Control	0.0 (0.0-1.5)	19	0.0 (0.0-2.5)	13	0.848
Case	0.0 (0.0-2.8)	42	1.8 (0.0-10.0)	34	0.026
Tumour in upper outer quadrant	0.0 (0.0-1.4)	18	2.3 (0.0-10.7)	29	0.008
Tumour in other quadrant	0.0 (0.0-3.2)	23	0.3 (0.0-1.6)	5	0.726

†Independent samples t-test with log10(x+1) transformed data. P-values < 0.01 indicate statistical significance (Bonferroni correction for multiple comparisons)

Discussion

The life style habit '*UCP application after shaving of underarm hair*' was rather equally distributed between cases and controls; two third of women separate this habit and one third uses a UCP after shaving. The risk of developing BC was increased by the self-reported life style habit '*UCP application after shaving'* but not significant.

Al absorption trough human skin after UCP application was intense discussed[22,68,69,112,135] but data about Al levels in bio-specimen of humans related to life-style habits including possible Al exposure are lacking[68]. This study presents comprehensive data on Al exposure via UCP application after shaving and incorporated Al. The significant relation of the habit to apply a UCP after shaving and higher Al levels in tissue and blood indicates that impaired human skin is more permeable for Al originating from UCPs than intact skin. Despite non-significant results, all Al levels of tissue and blood for controls, cases and subgroups of cases were higher for women who

applied a UCP after shaving (Table 8 and Table 9). These findings support the results of the recently published study that more frequent UCP use is related to higher Al levels in tissue[136] as well as the hypotheses of previous studies[22,24,69,73,74,133]. Al levels in tissue of controls showed a trend to be influenced by the habit, but due to high SDs and lower sample size the effect was not significant.

The effect of applying a UCP after shaving seems to be stronger on Al levels in blood than in tissue. Subgroup analysis showed that Al levels in blood of cases with a tumour in the upper outer quadrant were significantly higher for women who use a UCP application after shaving, while Al levels in blood of controls and cases with tumours in other quadrants were not influenced significantly by this habit. This stronger effect for women with a tumour in the upper outer quadrant was also observed in our previous study[136] and supports the hypothesis that especially tumours in the upper outer quadrant may be also induced by Al exposure via UCP application[75,76].

5.2.5 Self-reported physical activity and Al levels in tissue and blood

Physical activity has been consistently associated with cancer prevention[137,138] and with improved survival especially for younger women suffering from BC patients[139–141]. Presumably stored in tissues, toxic elements are identified also in perspiration and many toxic elements appeared to be preferentially excreted through sweat[142]. Recent studies suggest that perspiration is a major excretion route for systematic Al in humans[142,131,143]. The amount of perspiration strongly depends on time and intensity of physical activity[144]. Therefore it is hypothesized that more frequent physical exercise helps to lower Al levels in tissue and blood. The study questionnaire of the Innsbruck study on BC and Al included in addition to questions about UCP use also questions about the amount of physical exercise. Participants were asked to assign their general physical activity to one of the following categories: *'never', '1-4 times per month', '2-3 times per week'* up to *'4-7 times per week'*. For further statistical analysis these four classes were summarized to three categories: *'never-sometimes', 'regular' and 'often'*. Women suffering from BC were asked to categorise their physical activity prior to BC diagnosis. Especially in western societies it is common to apply a UCP also prior to physical exercise to prevent odour and strong sweating. To investigate a possible correlation of physical exercise and UCP use a Spearman correlation analysis was conducted. A Jonckheere-Terpstra trend test was performed on Al data of tissue and blood samples to detect a possible effect of physical exercise on incorporated Al levels.

Results

The self-reported physical activity was not significant different between women suffering from BC and healthy controls, χ^2 (2, N=159) = 2.73, p=0.255 (Table 10).

UCP use and amount of physical activity was negative but not significantly correlated for controls (r=-0.05, p=0.739, n=52), and for cases with a tumour in inner or lower quadrants (r=-0.1, p=0.457, n=45). Cases with a tumour in the upper outer quadrant showed a significant positive correlation of UCP use and physical activity (r=0.3, p=0.022, n=54).

Table 10 Physical exercise in breast cancer patients (cases) and healthy controls.

Physical exercise	Number of Cases (%) (n=106)	Number of Controls (%) (n=52)	Total	p-value (χ^2-test)
Never-sometimes	40 (37.7)	25 (47.2)	65 (40.9)	
Regular	42 (39.6)	14 (26.4)	56 (35.2)	0.255
Often	24(22.6)	14 (26.4)	38 (23.9)	

Self-reported physical activity compared to Al levels in **tissue**

Al levels in tissue of controls decreased significantly in women who reported to be more a more frequent physical active (p=0.036) but Al levels in tissue of cases not (p>0.05, Table 11, Figure 15A and B).

Table 11: Median (IQR) of Al levels in tissue (nmol/g dry weight) of cases and controls stratified by physical exercise.

Al levels in tissue	never to sometimes	n	regular	n	often	N	p-value†
Control	4.5 (3.0-6.1)	25	3.4 (2.0-5.3)	14	3.5 (1.9-3.9)	13	0.036
Case	5.7 (3.2-9.8)	38	7.0 (2.4-14.8)	39	5.2 (2.3-11.9)	20	0.893
Tumour in upper outer quadrant	5.3 (3.3-8.7)	17	8.9 (4.8-16.2)	26	5.8 (2.5-16.6)	11	0.367
Tumour in other quadrants	6.1 (3.4-9.8)	21	3.9 (1.2-12.7)	13	3.4 (1.9-7.6)	9	0.214

†Jonckheere-Terpstra trend test on medians of each sampling location.

Self-reported physical activity compared to Al levels in **blood**

Al levels in blood of controls decreased significantly in women who reported to be more frequent physical active (p=0.034) but Al levels in tissue of cases not (p=960, Table 12).

Table 12: Median (IQR) of Al levels in blood (µg/L) of cases and controls stratified by physical exercise.

Al levels in blood	never to sometimes	n	regular	n	often	N	p-value†
Control	1.5 (0.0-6.3)	15	0.0 (0.0-0.0)	8	0.0 (0.0-0.5)	0	0.034
Case	0.1 (0.0-9.4)	26	0.0 (0.0-3.1)	35	1.4 (0.0-3.4)	16	0.960

†Jonckheere-Terpstra trend test on medians of each sampling location.

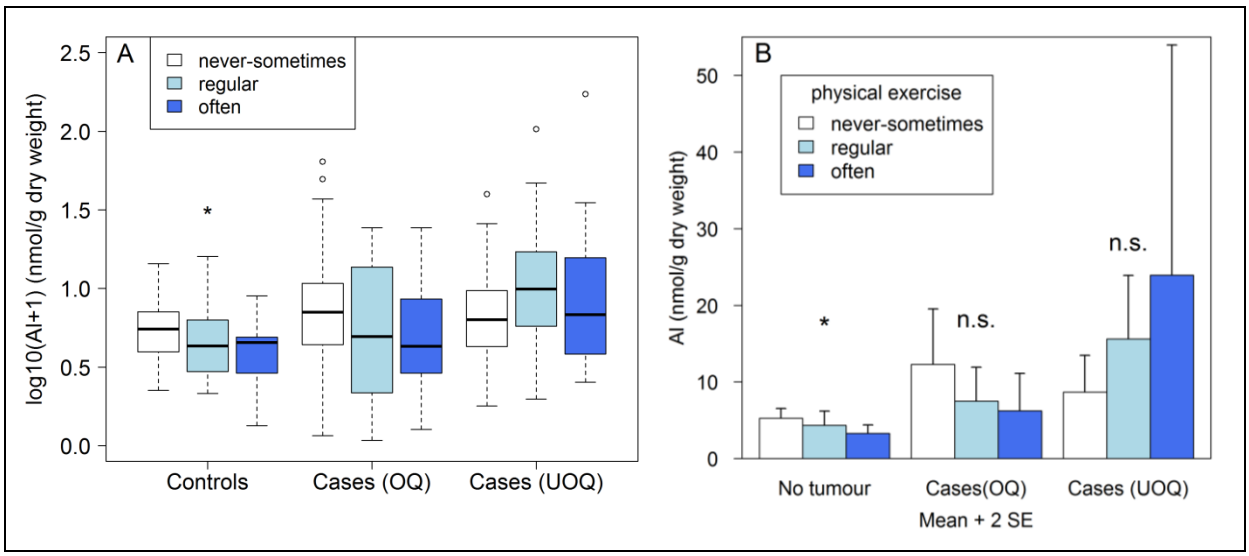

Figure 15 A) Boxplots of log10(x+1) transformed Al levels in tissue (nmol/g dry weight) of controls, cases with a tumour in lower and inner quadrants, expressed as other quadrants (OQ), and cases with an tumour in the upper outer quadrant (UOQ), splitted for three categories of physical activity. B) Barplot of untransformed Al levels in tissue (nmol/g dry weight) of controls and subgroup of cases, splitted for categories of physical exercise. Bars indicanting means and 2*SE, significant decrease of Al levels marked by an asteriks (**).

Discussion

The aim of this study part was to investigate the potential reduction of incorporated Al by self-reported physical activity. In contrast to cases, the results suggest that healthy women may be able to lower their Al levels in blood and tissue significantly by frequent physical exercise. These results support the findings of Minshall (2013) suggesting that sweating, increased by physical activity, is a major mechanism for the removal of systemic Al from the body [131].

There were no significant differences regarding self-reported physical activity between controls and cases.

Compared to healthy women, BC patients may be limited in their physical activity due to various side-effects of cancer therapies and due to the disease itself. Included women of the control group had also limitations in their physical activity because they underwent breast reduction surgery to gain less breast weight with the perspective to diminish back pain. Therefore an underrepresentation in the amount of physical exercise in cases and controls is likely and may the effect of physical activity/sweating could be even clearer in some healthy individuals. However, information on physical exercise where self-reported. Answers were categorised and later summarized over three life-time periods of the interviewed women and may include reporting bias.

Plotted data suggest that cases with tumours in lower and inner quadrants might be able to reduce Al levels in tissue by frequent physical exercise, but results were not significant. In contrast, cases with a tumour in the upper outer quadrant showed higher

Al levels in tissue in relation to frequent physical exercise, but these results were also not significant (Figure 15B). However it seems that cases with a tumour in the upper outer quadrant may incorporate more Al in tissue, even with more frequent physical exercise. For this subgroup the self-reported amount of physical exercise was significantly correlated to UCP use, suggesting that cases with a tumour in the upper outer quadrant likely used more frequent UCPs in relation to more frequent physical activity. These preliminary findings and the significant positive correlation of UCP application and physical exercise, suggest that women with a tumour in the upper outer quadrant may increase their body burden due to two mechanisms: first, they may do not excrete Al due to blocked or inactivated sweat glands by UCP use[30,133,131] and second, they may deliver additional Al due to UCP application[136].

If sweating, is the major route for the removal of systemic Al from the body, then this findings raise even more doubts concerning the practise of blocking perspiration using antiperspirants with or also without Al particularly before physical exercise[131].

6. Final conclusion and outlook

This work relates comprehensive data of life-style habits to Al levels in bio-specimen. The major findings of the Innsbruck study on BC and Al were that frequent use of UCPs may lead to accumulation of aluminium in breast tissue and that extensive use of UCPs particularly at young age, was associated with increased risk of BC. Considering these results together with the additional results that more frequent use of UCPs is related to an earlier diagnosis of BC, that shaving of armpit hair led to higher circulating and incorporated Al and that physical exercise could help to reduce Al due to sweating, the practice of disrupting or blocking perspiration using Al-based UCPs is getting even more equivocal.

Mandriota et al., (2016) showed that Al concentrations in the range of those measured in the human breast transform cultured mammary epithelial cells on the whole to form tumours and metastases in mouse cancer models[23]. The Innsbruck study on BC and Al showed that Al levels in human breast tissue were related to UCP use and that several life style habits may diminish this possible carcinogenic level of Al in breast tissue.

In spite of the fact that physical exercise is a prevention factor for breast cancer[137,145,146] the previous findings about Al excretion in sweat[131], and the latest findings about physical exercise, UCP use and Al levels in bio-specimen may

additionally explain why physical exercise contributes to health in general and especially to women's health.

Many studies have reported adverse effects associated with incorporated Al levels and novel findings of the Innsbruck case-control study on Al and BC support the hypothesis of higher Al exposure contributing to the aetiology of BC development. This study based on self-reported data about Al exposure of a relatively small population of 418 women and Al levels in bio-specimen of even less donors. Moreover the study results are based on correlation analyses and not on causal links. Hence for definitive answers in-depth studies with detailed data about Al exposure, individual life-style habits and comprehensive bio-specimen analysis together with analysis about DNA abbreviation and mutations are necessary.

Beside the fact that the highly useful metal Al possibly contributes to various NCDs the individual exposure of Al through nutrition and cosmetics like Al-based antiperspirants is avoidable. Environmental pollution is a large, costly, inequitably distributed but a preventable cause of disease and death in countries around the world. The links between pollution and health though very strong, have been insufficiently appreciated in the health agenda of individuals, societies and health ministries[10]. Considering the human impact on bioavailability of Al there is evidence that Al has entered the biotic cycle. It seems that Al is accumulating in the biosphere and there is less prospect of a quick return in the lithospheric cycle[30]. Although Al exposure of consumers is not already revised by authorities, informed consumers are able to minimize their Al exposure during their every-day life.

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Appendix

Full paper

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Research Paper

Use of Underarm Cosmetic Products in Relation to Risk of Breast Cancer: A Case-Control Study

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ABSTRACT

Background: Previous studies on breast cancer (BC), underarm cosmetic products (UCP) and aluminum salts have shown conflicting results. We conducted a 1:1 age-matched case-control study to investigate the risk for BC in relation to self-reported UCP application.
Methods: Self-reported history of UCP use was compared between 209 female BC patients (cases) and 209 healthy controls. Aluminum concentration in breast tissue was measured in 100 cases and 52 controls. Multivariable conditional logistic regression analysis was performed to estimate odds ratios (ORs) with 95% confidence intervals (CIs), adjusting for established BC risk factors.
Findings: Use of UCP was significantly associated with risk of BC ($p = 0.036$). The risk for BC increased by an OR of 3.88 (95% CI 1.03–14.66) in women who reported using UCP's several times daily starting at an age earlier than 30 years. Aluminum in breast tissue was found in both cases and controls and was significantly associated to self-reported UCP use ($p = 0.009$). Median (interquartile) aluminum concentrations were significantly higher ($p = 0.001$) in cases than in controls (5.8, 2.3–12.9 versus 3.8, 2.5–5.8 nmol/g).
Interpretation: Frequent use of UCPs may lead to an accumulation of aluminum in breast tissue. More than daily use of UCPs at younger ages may increase the risk of BC.

1. Background

Breast cancer is the most common cancer in women with a high prevalence in economically developed countries (Kristensen et al., 2014; Parkin et al., 2005). The etiology of breast cancer is multifactorial. Age, genetic mutations and life-time estrogen exposure are well known risk factors (Gail and Pfeiffer, 2015; Petracci et al., 2011; Pfeiffer et al., 2013). These factors explain only a small part of the etiology (Turnbull and Rahman, 2008) suggesting that environmental factors may also be relevant in the development of breast cancer (Bonefeld-Jorgensen et al., 2011; Coyle, 2004). A change in the topological distribution of mammary carcinoma since 1975 (Bright et al., 2016; Darbre, 2016, 2009, 2005, 2003) towards an higher incidence in the upper outer quadrant seems to point to underarm cosmetic products (UCPs) as a potential contributor (Darbre, 2009, 2005, 2003; Darbre et al., 2013b). Previous studies investigating the effect of UCPs on breast cancer have shown conflicting results (McGrath, 2003; Mirick et al., 2002; Pasha et al., 2008; Rodrigues-Peres et al., 2013). Therefore, latest systematic reviews were not able to provide conclusive evidence (Namer et al., 2008; Willhite et al., 2014). Active ingredients in most UCPs are aluminum-based compounds as aluminum chloride and aluminum chlorohydrate. Aluminum salts have been associated with oxidative stress, DNA double strand breaks, proliferation, interference in estrogen action before (Darbre, 2009; Darbre et al., 2013a; Dyrssen et al., 1987; Farasani and Darbre, 2015; Lankoff et al., 2006; Sappino et al., 2012) and with metastasis recently (Mandriota et al., 2016). Mandriota et al. (2016a) demonstrated in an established cancer mouse model that concentrations of aluminum in the range of those measured in human breast are able to transform cultured mammary epithelial cells, enabling them to form tumors and to metastasize. It was further suggested that frequent use of UCPs containing aluminum salts is a main source of measured aluminum in breast structures (Darbre et al., 2013b, 2011; Exley et al., 2007; Mannello et al., 2009). Due to the genotoxic and possibly

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carcinogenic effect of aluminum salts, the use of UCPs may be related to breast cancer (Darbre, 2001; Jennrich and Schulte-Uebbing, 2016; Pineau et al., 2014; Rodrigues-Peres et al., 2013; Sappino et al., 2012).

The relationship of UCPs containing aluminum salts with breast cancer was investigated in few epidemiological studies showing conflicting results (Fakri, 2006; McGrath, 2003; Mirick et al., 2002). Mirick et al. (2002) and Fakri (2006) found no significant associations between antiperspirants and increased risk of breast cancer. In contrast, McGrath (2003) found that patients using UCPs frequently received their breast cancer diagnosis at an earlier age than patients avoiding UCPs. However, none of these studies included breast tissue measurements of aluminum with regard to UCP use. There exists, so far, no controlled study investigating the relationship of aluminum with breast cancer combining an epidemiologic approach with breast tissue measurements.

We conducted a 1:1 age-matched hospital-based case-control study aiming to investigate the risk for breast cancer in relation to self-reported UCP use. We included measurements of aluminum concentrations in breast tissue from a large series of breast cancer patients and healthy individuals in a controlled epidemiologic study. We hypothesized that (1) breast cancer patients had used UCPs more frequently during their lives than healthy controls, that (2) aluminum concentrations in breast tissue is increased in cases, and that (3) there is a relationship between UCP use and measured aluminum concentrations in breast tissue.

2. Methods

2.1. Study Design and Participants

Participants of this age-matched case-control study were recruited between January 2013 and October 2016 at the Medical University of Innsbruck, Austria. Eligible cases were all breast cancer patients aged 20–85 years treated by the Department of Obstetrics and Gynecology who had a confirmed diagnosis of breast cancer within the last 5 years. Eligible controls were women in the same age range (±2.5 years) without a history of malignant breast disease. Controls were recruited either at the Department of Plastic, Reconstructive and Aesthetic Surgery or at other departments. Selection of controls did not follow a formal probability sampling scheme. Because of organizational limitations sampling was done on random time points when trained interviewers were available to find voluntary women fulfilling the inclusion criteria. Cases undergoing mastectomy and healthy controls undergoing reduction mammoplasty were eligible for tissue sampling.

The study was approved by the ethics committee of the Medical University of Innsbruck, (UN4759, 315/4.6). All participants provided their written informed consent before taking part in the study.

2.2. Data Source and Tissue Samples

2.2.1. Structured Personal Interview

A structured personal interview was performed with all study participants by interviewers who were trained to avoid suggestive questions and to use the key words antiperspirants, deodorants and aluminum very carefully. The interviewers were medical school students in their last year and a graduated psychologist. The questionnaire used in these interviews was a modified version of the validated questionnaire used in the MARIE study (Slanger et al., 2007). Study participants were blinded as to the purpose of the study. They were asked to attend a study on life style factors and BC, including questions about nutrition, physical activity and personal hygiene. There was no special focus on UCP use. We also collected information on other BC related characteristics such as estrogen and hormone exposure as well as genetic factors. Questions asked refer to past exposure in four lifetime categories: 'under the age of 30 years', 'between 30 and 50 years', 'over the age of 50 years' and 'last five years before breast cancer diagnoses'. We extended this questionnaire by specific questions regarding personal hygiene, UCP use and aluminum exposure. The majority of UCPs on the market during the past years were antiperspirants containing aluminum salts as active ingredients. There are a few UCPs without aluminum salts commonly called "deodorants" containing ingredients such as perfumes and etheric oils. When asked it turned out that most women were not able to discriminate between these two kinds of UCPs. We therefore concluded that it would be misleading to analyze antiperspirants and deodorants separately and consequently summarized them into the term UCP as the main exposure variable. UCP application categorized in "never", "1–4 times per month", "2–6 times per week", "daily" and "several times per day" was defined as the primary endpoint of this study.

2.2.2. Tissue Sampling and Measurement

Tissue sampling was performed in all cases and controls undergoing surgery. In cases, we took samples of the breast affected by the tumor, in controls sampling was performed on both breasts. Samples of 500 mg were collected near the axilla in the upper outer quadrant, near the mammilla and near the lateral sternal edge in the lower inner quadrant. Thus, we collected three samples in cases and six samples in controls.

In cases, breast tissue was sampled at the day of surgery at the Morphology Laboratory of the Department of Obstetrics and Gynecology during preparation for macroscopic and histo-pathological analysis. In controls, tissue sampling was performed during the breast reduction surgery in the operation theatre of the Department of Plastic, Reconstructive and Aesthetic Surgery. Samples were carefully collected avoiding any background contamination with aluminum regarding the use of surgical instruments, lab tools and vials. Samples were labelled with a patient code blinding any information regarding case/control assignment and tissue location and were immediately frozen and stored at −80 °C at the Department of Biochemistry until analysis. Tissue preparation and defatting was conducted as described in Exley et al. (2007). In brief, thawed tissue was defatted by incubation at 37 °C for maximal 72 h to assure that dried tissue achieved constant weight. Mean of wet weight of samples was 400 mg (±100 mg), mean of dried tissue was 150 mg (±100 mg). Fat was released as clear oil during drying process in inclined plastic weighing boats. For degreasing and tissue transfer we only used metal free instruments. Dry, weighed and defatted tissue was transferred into 20 mL PFA Teflon© vessels with venting plugs and screw caps (CEM Microwave Technology, Germany). Further tissue preparation, digestion and dilution were done according to House et al., 2013. For digestion we used high quality Nitric acid 69% Trace SELECT® (Sigma-Aldrich, Germany). Digested and diluted tissue samples as well as ninety method blanks were analyzed as clear fluids with graphite furnace atomic absorption spectrometer (GF-AAS) with Zeeman–effect background corrector (Thermo Scientific, Germany).

2.3. Statistical Analysis

The sample size of this case-control study was pre-specified and determined to be adequate to detect an odds ratio (OR) of 2 or greater for UCP application on a significance level of 5%.

Assuming a control proportion of 65% UCP use as in Mirick et al. (2002), to achieve 80% statistical power, we were aiming to recruit 200 participants per group, a total of 400 women. In total we recruited 460 participants, 210 cases and 250 controls. Each case was age-matched in a 1:1 ratio to one control subject, minimizing the age difference within case-control pairs by a validated matching algorithm. The application of this algorithm ensured an objective and random assignment of cases to controls in order to reach the optimum result in terms of age difference. Consequently, the pairs differed regarding interview dates.

Patient characteristics, genetic factors, hormone exposure, life style parameters, UCP use were compared between cases and controls using descriptive statistics. Means and medians as well as standard deviations (SD) and interquartile ranges (IQR) were calculated to summarize continuous variables. Categorical variables were presented as frequencies and percentages. We conducted conditional logistic

regression analyses to determine relative risks, estimated as odds ratios (ORs) with 95% confidence intervals (CI) for UCP application and other exposures related to breast cancer. The final multivariable model included all variables that showed a p-value < 0.25 in univariable analyses as well as all relevant variables known to be associated with breast cancer (Pfeiffer et al., 2013). We assessed effect modification through tumor localization and timing of interviews by including interaction terms into the adjusted conditional logistic regression models.

Aluminum concentrations from the different sampling locations (three per case and six per control) were averaged per women, summarized with medians and interquartile ranges (IQR) for cases and controls and stratified by UCP application. In a first step, the summarized aluminum concentrations were compared between cases and controls with an independent t-test. In a second step, a three-way ANOVA for repeated measurements with the between-subject factor 'case versus control', 'UCP use' as ordinal scaled covariate, and the within-subject factor 'sampling location' was performed on $\log10(x + 1)$ aluminum concentrations. We performed subgroup analysis for aluminum measurements separately for cases with tumors in the upper outer quadrant and tumors in other quadrants. We considered a p-value smaller than 0.05 as statistically significant. For both matching and statistical analysis SPSS Statistics v.22 (IBM Analytics, Armonk, NY, USA) was used.

3. Results

A total of 460 women participated in this study, of these 210 were breast cancer cases and 250 were healthy controls. We excluded one case due to breast cancer diagnosis earlier than 5 years before the interview. One control had to be excluded due to unclear breast tissue pathology. Finally, 209 cases were matched 1:1 to 209 controls minimizing the age differences within pairs to a maximum of 3.5 years. Consequently cases and controls did not differ regarding mean age (51.9 ± 12.0 versus 51.8 ± 12.1). Tissue samples were available in 100 cases and 52 controls undergoing surgery.

Characteristics of breast cancer patients and healthy controls together with crude ORs from univariable analyses are shown in Table 1. As expected positive family history of breast cancer was the most pronounced risk factor. Further characteristics that were significantly different between cases and controls were a family history of other cancers such as prostate, ovarian and endometrium cancer, history of benign breast disease and a lower body mass index.

As shown in Table 2, self-reported use of UCP at early ages (<30 years) was significantly associated with an increased risk of breast cancer ($p = 0.0358$) adjusting for age, family history of breast cancer, family history of other cancer, history of benign breast disease, age at menarche, parity, age at birth of first child, age at menopause, menopausal status, hormone replacement therapy, average body mass index and alcohol consumption. This association was triggered by women who reported that they had used UCPs several times per day under their age of 30 increasing their risk for breast cancer by an OR of 3.88 with a 95% CI of 1.03–14.66 ($p = 0.0456$).

Aluminum in breast tissue (Table 3) was found in both cases and controls ranging from 0 to 367.38 nmol/g dry weight and was significantly associated with self-reported UCP use ($p = 0.0344$ for UCP use under the age of 30, $p = 0.0093$ for UCP use during the last 5 years). In cases, median (interquartile) aluminum concentrations observed were 5.8 (2.3–12.9) nmol/g, significantly higher ($p = 0.0014$) than in controls (3.8, 2.5–5.8 nmol/g).

In addition, we analyzed whether tumor localization modifies the relationship between self-reported UCP use, aluminum concentration and the risk for BC. Regarding UCP use there was no significant effect modification by tumor localization ($p = 0.680$ for the UCP use <30 years, $p = 0.341$ for the UCP use during last 5 years). In contrast, regarding measured aluminum concentrations, the stratified results for tumor localization showed significant differences between cases and controls in the subgroup of cases with a tumor in the upper outer quadrant only (Table 4).

4. Discussion

The findings of this age-matched hospital based case-control study suggest an association between UCP use, aluminum concentration in breast tissue and breast cancer. We found a significant difference between cases and controls in the pre-specified primary endpoint.

Table 1
Self-reported characteristics of breast cancer patients and healthy controls.

	Cases ($n = 209$)	Controls ($n = 209$)	Crude OR (95% CI)[a]	p-Value
Age at interview [years, means (SD)]	51·9 (12·0)	51·8 (12·1)		0·2994
Family history of breast cancer (%)	76 (36·4)	32 (15·3)	2·91 (1·81–4·68)	<0·0001
None	133 (63·6)	177 (84·7)	Reference	
1 person	48 (23·0)	27 (12·9)	2·21 (1·30–3·74)	0·0034
2 or more	28 (13·4)	5 (2·4)	6·31 (2·4–6·53)	0.0002
Family history of other cancer (%)	128 (61·5)	103 (49·3)	1·60 (1·09–2·35)	0·0176
History of benign breast disease (%)	63 (30·1)	43 (20·6)	1·61 (1·04–2·48)	0·0326
Age at menarche [years, means (SD)]	13·5 (1·7)	13·4 (1·5)	1·04 (0·92–1·17)	0·5547
Menstruation (%)				
Regular	164 (78·5)	171 (81·8)	Reference	
Unregularly	42 (20·1)	37 (17·7)	1·19 (0·71–1·98)	0·5155
Unknown	3 (1·4)	1 (0·5)		
Hormonal contraceptives (%)	164 (78·5)	168 (80·4)	0·87 (0·52–1·46)	0·5997
Parity (%)	176 (84·2)	172 (82·3)	1·17 (0·68–2·01)	0·5794
Age at birth of first child [years, means (SD)]	26·1 (5·6)	25·1 (5·3)	1·02 (0·98–1·08)	0·3838
Lactation (%)	137 (65·6)	132 (63·5)	1·09 (0·73–1·61)	0·6861
Lactation [months, means (SD)]	3·8 (4·5)	4·0 (5·3)	0·99 (0·95–1·03)	0·7033
Age at menopause	47·3 (7·2)	48·6 (5·7)	0·98 (0·93–1·03)	0·2990
Hormone replacement therapy (%)	44 (21·1)	34 (16·3)	1·42 (0·84–2·39)	0·1881
Average body mass index [kg/m^2, means (SD)]	22·8 (3·4)	23·4 (4·0)	0·95 (0·89–0·99)	0·038
Smoking (%)				
Never	100 (47·8)	98 (46·9)	Reference	
Sometimes	20 (9·6)	28 (13·4)	0·70 (0·37–1·32)	0·2733
Regular	89 (42·6)	83 (39·7)	1·07 (0·69–1·66)	0·7586
Alcohol consumption (%)				
0 drinks per day	29 (13·9)	29 (14·0)	Reference	
≤1 drink per day	172 (82·3)	175 (84·5)	1·02 (0·58–1·82)	0·9364
1+ drink per day	8 (3·8)	3 (1·4)	2·72 (0·65–11·34)	0·1684

[a] Derived from univariable conditional logistic regression analysis.

Table 2
Use of underarm cosmetic products (UCP) in breast cancer (BC) patients and healthy controls.

	Number of cases (%) ($n = 209$)	Number of controls (%) ($n = 209$)	Crude OR (95% CI)	Crude *p*-value	Adjusted OR[a] (95% CI)	Adjusted *p*-value
UCP use in women when they were under the age of 30				0·0951		0·0358
Never	43 (20·6)	46 (22·0)	Reference		Reference	
1–4 times per month	19 (9·1)	26 (12·4)	0·83 (0·40–1·73)	0·6222	0·50 (0·20–1·26)	0·1435
2–6 times per week	26 (12·7)	36 (17·2)	0·87 (0·43–1·75)	0·6930	0·53 (0·23–1·25)	0·1486
Daily	103 (49·3)	89 (42·6)	1·40 (0·79–2·53)	0·2603	1·03 (0·51–2·07)	0·9390
Several times per day	18 (8·6)	9 (4·3)	2·84 (1·02–7·89)	0·0451	3·88 (1·03–14·66)	0·0456
Unknown	0 (0·0)	3 (1·4)				
UCP use during last 5 years before BC diagnosis in cases/during last 5 years before interview in controls				0·1104		0·0822
Never	25 (12·0)	34 (16·3)	Reference		Reference	
1–4 times per month	24 (11·5)	21 (10·0)	1·67 (0·73–3·81)	0·2211	1·41 (0·49–4·04)	0·5216
2–6 times per week	31 (14·8)	45 (21·5)	0·99 (0·49–2·02)	0·9824	0·59 (0·25–1·40)	0·2338
Daily	109 (52·2)	96 (45·9)	1·70 (0·90–3·21)	0·1046	1·22 (0·56–2·66)	0·6105
Several times per day	20 (9·6)	13 (6·2)	2·63 (1·00–6·87)	0·0492	3·16 (0·90–11·15)	0·0736
Unknown	0 (0·0)	0 (0·0)				

[a] Adjusted for age at interview, age at menarche, parity, age at first live birth, menopausal status, age at menopause, MHT drug therapy, history of breast cancer, history of benign breast disease, family history of other cancer, BMI, alcohol consumption in multivariable conditional logistic regression analysis.

However, the observed association of UCP use with breast cancer was in fact limited to women who reported using UCP's several times a day when they were under the age of 30.

In contrast to our findings, previous epidemiologic studies (Fakri, 2006; Mirick et al., 2002) did not support the hypothesis that UCP use increases the risk for breast cancer. Fakri (2006) examined a very small sample of 54 unmatched cases and 50 controls underpowered to detect realistic effect sizes. In their study UCP use was dichotomous categorized in just two levels, using of UCPs versus no use, which is too imprecise in regard to our results, where a significant association was observed only when women used UCPs several times per day. Similarly, in the much larger study of Mirick et al. (2002), UCP use was measured also in a dichotomous way only. In the study of Mirick et al. (2002) study participants were not asked about UCP use in different life time categories and therefore possible effects of UCP use at younger ages were not detectable. In fact, Mirick et al. (2002) reported antiperspirant use rather than UCP use, however, in the light of our experiences it is unclear how the authors discriminated between deodorant and antiperspirant use. Another important difference between Mirick et al. (2002) and our study exists regarding the birth cohorts of breast cancer patients recruited into the two studies. Breast cancer patients participating in the study of Mirick et al. (2002) were diagnosed in the early 1990′s, on average 20 years earlier than patients in our study. At the time relevant for exposure, approximately between 1940 and 1960, the use of UCPs was less common than 20 years later. UCP use strongly increased in the last four decades and also cultural habits such as shaving of axilla hair became only popular during the late 1980′s in western countries (Darbre, 2009, 2003; McGrath, 2003).

So far, there exist six studies that measured aluminum concentration in breast cancer patients comparing concentrations between benign and malign breast tissues (Exley et al., 2007; House et al., 2013; Millos et al., 2009; Ng et al., 1997; Pasha et al., 2008; Rodrigues-Peres et al., 2013). These studies differed considerably regarding the amount of aluminum found in breast tissue likely because of discrepancies in measurement techniques. Regarding, the analytical approach the measured aluminum concentrations in our cohort were similar to the studies of House et al. (2013) and Rodrigues-Peres et al. (2013).

None of the previous studies sampled control tissue from healthy individuals. Our study included tissue measurements of breast cancer patients and healthy individuals observing a significant difference

Table 3
Median (IQR) of total aluminum concentrations [nmol/g dry weight] in breast tissue samples of cases and controls stratified by underarm cosmetic product (UCP) use.

	Cases	n	Controls	n	*p*-value sampling location	*p*-value UCP use	*p*-value cases vs controls
Median (IQR) of Al^{3+} concentration[a]	5·77 (2·29–12·90)	100	3·77 (2·47–5·78)	52			0·0014
UCP use in women when they were under the age of 30[b]							
Never	3·58 (1·72–9·25)	28	2·74 (1·90–4·21)	11	0·100	0·0344	0·0269
Several times per week	7·77 (4·74–11·40)	9	3·07 (2·75–4·52)	4			
Daily	6·07 (2·21–14·89)	53	4·34 (2·67–6·42)	34			
Several times per day	11·29 (3·62–13·21)	9	2·51 (1·86–4·86)	3			
UCP use during last 5 years before BC diagnosis in cases/during last 5 years before interview in controls[b]							
Never	3·58 (1·72–7·32)	20	3·32 (1·90–4·21)	10	0·251	0·0093	0·0376
Several times per week	7·74 (3·23–11·40)	10	3·07 (2·55–5·86)	6			
Daily	6·07 (2·34–14·89)	57	3·96 (2·54–5·99)	31			
Several times per day	12·10 (3·50–14·68)	12	4·86 (2·51–10·23)	5			

[a] Independent samples *t*-test with log10(x + 1) transformed data.
[b] Three-way analysis of variance with log10(x + 1) transformed data. Repeated aluminum measurements at three different sampling locations (upper outer, mammilla and lower inner breast quadrant) were considered as within-subject factor in the ANOVA.

Table 4
Median (IQR) of total aluminum concentrations [nmol/g dry weight] in breast tissue samples of cases and controls stratified by underarm cosmetic product (UCP) use. Subgroup analyses for cases with tumors in the upper outer quadrant (a) and for cases with tumors in other quadrants (b).

	Cases	n	Controls	n	*p*-value sampling location	*p*-value UCP use	*p*-value cases vs controls
a) Tumor located in the upper outer quadrant							
Median (IQR) of Al^{3+} concentration[a]	7·00 (3·10–16·15)	55	3·77 (2·47–5·78)	52			0·0003
UCP use in women when they were under the age of 30[b]							
Never	3·43 (1·55–9·69)	14	2·74 (1·90–4·21)	11	0·757	0·0116	0·0028
Several times per week	7·71 (4·74–7·77)	5	3·07 (2·75–4·52)	4			
Daily	8·35 (3·19–24·87)	31	4·34 (2·67–6·42)	34			
Several times per day	12·25 (8·56–14·68)	4	2·51 (1·86–4·86)	3			
UCP use during last 5 years before BC diagnosis in cases/during last 5 years before interview in controls[b]							
Never	3·09 (1·55–5·34)	10	3·32 (1·90–4·21)	10	0·916	0·0079	0·0054
Several times per week	7·71 (3·27–7·77)	5	3·07 (2·55–5·86)	6			
Daily	7·69 (3·59–18·41)	32	3·96 (2·54–5·99)	31			
Several times per day	12·90 (3·83–16·15)	7	4·86 (2·51–10·23)	5			
b) Tumor located in other quadrants							
Median (IQR) of Al^{3+} concentration[a]	3·94 (1·90–10·92)	45	3·77 (2·47–5·78)	52			0·2642
UCP use in women when they were under the age of 30[b]							
Never	4·63 (1·90–8·82)	14	2·74 (1·90–4·21)	11	0·017	0·3457	0·3558
Several times per week	11·16 (7·08–16·18)	4	3·07 (2·75–4·52)	4			
Daily	3·48 (1·24–8·99)	22	4·34 (2·67–6·42)	34			
Several times per day	3·62 (3·39–12·91)	5	2·51 (1·86–4·86)	3			
UCP use during last 5 years before BC diagnosis in cases/during last 5 years before interview in controls[b]							
Never	4·63 (1·90–7·91)	10	3·32 (1·90–4·21)	10	0·015	0·1316	0·3731
Several times per week	10·92 (3·23–11·40)	5	3·07 (2·55–5·86)	6			
Daily	3·94 (1·51–9·76)	25	3·96 (2·54–5·99)	31			
Several times per day	3·62 (3·39–12·91)	5	4·86 (2·51–10·23)	5			

[a] Independent samples *t*-test with log10(x + 1) transformed data.
[b] Three-way analysis of variance with log10(x + 1) transformed data. Repeated aluminum measurements at three different sampling locations (upper outer, mammilla and lower inner breast quadrant) were considered as within-subject factor in the ANOVA. *P*-values < 0.0125 (Bonferroni correction for multiple comparisons) indicate statistical significance.

regarding aluminum concentrations. Beyond this, we were able to show a significant association between measured aluminum concentrations in breast tissue and self-reported UCP use suggesting dermal absorption of aluminum salts.

Differences in aluminum concentration between cases and controls were only evident when restricting the analysis to cases with tumors in the upper outer quadrant, supporting the hypothesis of Darbre (2005, Darbre, 2009) that tumors in the upper outer quadrant are affected by the use of UCPs. Results of the questionnaire part, however, do not support this hypothesis. Self-reported UCP use did not differ significantly between cases and controls when considering tumor localization.

Tissue samples of controls showed less variation in aluminum concentrations than samples of breast cancer patients. In ten breast cancer patients, aluminum concentrations over 60 nmol/g up to 367 nmol/g dry weight (15–115 nmol/g wet weight) were observed. Mandriota et al. (2016) and colleges recently showed that aluminum salt concentrations of 100 nmol/g wet weight lead to transformation of in-vitro cultured mammary epithelial cells enabling them to form tumors and metastasis in mouse models. In contrast, aluminum concentration in controls reached a maximum of 24.5 nmol/g dry weight (8 nmol/g wet weight) only.

Our study has several strengths. We combined comprehensive questionnaire data of breast cancer cases and healthy individuals on underarm hygiene habits with data of aluminum concentration in tissue samples. We applied a well-developed and accurate method for aluminum measurement (Exley et al., 2007; House et al., 2013). A standardized sampling procedure, high purity of reagents and a high measurement accuracy minimized background contamination. It is likely that aluminum in breast tissue has a patchy distribution (Exley et al., 2007; House et al., 2013), therefore, we collected multiple tissue samples alongside the transect from upper outer to upper inner quadrant.

Certain limitations of our study need to be discussed. A case-control study is susceptible to recall bias. Self-reporting information may be incomplete or inaccurate and may differ between cases and controls. Younger women may remember in more detail about their specific hygiene habits than elderly women. The mix of incident and prevalent cases in our study may be an additional source of bias. We assessed whether the time span between BC diagnosis and interview date is an effect modifier for the relation of UCP use with risk for BC. Although there is no significant effect modification of the different timing of interviews ($p = 0.282$, for the 'UCP use under the age of 30' model, $p = 0.877$ for the 'UCP use in the last 5 years' model) we cannot rule out any recall issues between incident and prevalent cases.

We tried to reduce reporting and measurement bias by performing personal interviews with well-trained interviewers. The limited sample size of the study leads to relatively small numbers in the sub-categories of the main exposure variable. Though significant, the result concerning UCP use several times per day is based on a few cases only. Furthermore, we cannot exclude a reverse causation effect, meaning that the breast tumor may accumulate aluminum. There are studies that reported higher levels of transition metals in tissue of breast cancer patients (Cui et al., 2007; Ionescu et al., 2006; Romanowicz-Makowska et al., 2011). Although, we matched cases and controls on age, the subgroup for tissue sampling is not age matched. However, in our study, aluminum concentrations did not correlate with age ($r = -0.028$, $p = 0.7291$).

In conclusion, our study provides novel insights and additional evidence regarding a possible role of UCP use and aluminum salts in the etiology of breast cancer. Our findings suggest that frequent use of

UCPs may lead to an accumulation of aluminum in breast tissue. We could show that women who reported to use UCPs several times a day starting at an age under 30 years may even have an increased risk for breast cancer. Until definitive answers about the involvement of aluminum in carcinogenesis of breast cancer, we recommend that particularly women at their younger ages should be careful with the use of UCPs and avoid its excessive use.

Collaborators

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Conflicts of Interest

All authors declared no conflicts of interest.

Author Contributions

HU, NC and CL designed the study and wrote the protocol. CL and HU wrote the first draft of the manuscript. CL conducted interviews, did tissue collection and preparation. CE trained CL for tissue preparation and tissue digestion. HT supervised the tissue analysis. CL did the data management and data analysis with the supervision of HU. NC organized study conduct at the Department of Obstetrics and Gynecology. AS, DG and SS performed macro analysis of breast mastectomies. HF supplied laboratory infrastructures for tissue storage and gave organizational support. ST, TC, DE and MH recruited breast cancer patients and performed mastectomies. TB conducted breast reduction surgeries. EMM, JK, CB, SR, FW, DP, FM, TI recruited healthy controls and did interviews. HHL supplied all laboratory infrastructures for tissue analysis and gave his experienced support. All authors were involved in revision of the final manuscript.

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Abstract I (accepted)

Linhart C, Talasz H, Morandi EM, Exley C, Lindner HH, Taucher S, et al. Use of underarm cosmetic products and breast cancer: a case-control study. ***Int J Gynecol Cancer*** **2017,** (poster presentation at the International Meeting at the European Society of Gynaecological Oncology (ESGO), Vienna, 2017.)

Use of underarm cosmetic products and breast cancer: a case-control study

Caroline Linhart[1], Heribert Talasz[2], Evi M. Morandi[3], Christopher Exley[4], Herbert H. Lindner[2], Susanne Taucher[5], Daniel Egle[5], Michael Hubalek[5], Theresa Czech[5], Heidi Fiegl[5], Afschin Soleiman[5], Thomas Bauer[3], Nicole Concin[5*], Hanno Ulmer[1*]

* corresponding authors

Objectives: We conducted a 1:1 age-matched case-control study to investigate the risk for breast cancer (BC) in relation to self-reported use of underarm cosmetic products (UCPs) containing aluminium salts. Our study for the first time also included analysis of aluminium concentrations in a big series of breast tissues.

Methods: BC risk interviews were conducted. History of UCP use was compared between 209 BC patients (cases) and 209 age-matched healthy women (controls). Aluminium concentration was analysed in breast tissues of 100 cases and 52 controls that underwent mastectomy for BC or reduction mammoplasty for non-cancer reasons, respectively. Multivariable conditional logistic regression analysis was performed to determine relative risks, estimated as odds ratios (ORs) with 95% confidence intervals (CIs), adjusting for established BC risk factors.

Results: Case-control comparisons confirmed established risk factors for BC. Self-reported use of UCPs? was significantly associated with an increased risk of BC (p=0.036). BC risk increased by an OR of 3.88 (95% CI 1.03-14.66) in women who reported using UCPs more than once daily starting at an age <30. Aluminium in breast tissue was significantly associated to self-reported UCP use (p=0.003) in both cases and controls. Median (interquartile) aluminium concentration was significantly higher (p<0.001) in cases than in controls (5.8, 2.3-13.1 versus 3.8, 2.5-5.8 nmol/g).

Conclusions: Frequent use of UCPs may lead to accumulation of aluminium in breast tissue. Extensive use of UCPs particularly at young age was associated with increased risk of BC. Off the record, we report on pure correlation analyses and not on causal links.

Abstract II - abstract VI

Additional Poster 14

Antiperspirants with aluminium salts and the relation to breast cancer.

Caroline Linhart[a]*, Nicole Concin[b], Susanne Taucher[b], Herbert Lindner[c], Hanno Ulmer[a]

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Studies of antiperspirants containing aluminium salts and their effect on breast cancer have shown conflicting results. We designed a study consisting of two parts.
Case-control study: History of antiperspirant use will be compared between a group of 262 female breast cancer patients aged 20–85 years (n=131 cases and) and age-matched controls (n=131) without breast cancer. A personal interview regarding individual hygiene, life-style, and aluminium exposure will be performed. The study questionnaire is partly based on the MARIE study of the German Cancer Research Centre.

Cohort study approach: A total of 100 consecutive patients requiring breast biopsy will be recruited and different tissue parts of the breast region (axilla, lateral, middle, medial, fat, and connective tissue) will be collected for determination of aluminium with atomic absorption spectroscopy (AAS). Also, these patients will be interviewed. Results will be compared between patients with subsequent breast cancer diagnosis and patients with benign outcome.

The use of antiperspirants containing aluminium-salts and its relation to breast cancer: Methods and implementation of biospecimen sampling

Dominik Panosch[1], Franziska Weidenbeck[1], Michael Hubalek[2], Evi Morandi[3], Herbert Lindner[4], Heribert Talasz[4], Christopher Exley[5], Nicole Concin[2], Hanno Ulmer[1], Caroline Linhart[1]

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Recent publications suggest that breast cancer is linked to the use of antiperspirants containing aluminium-salts. We designed a hospital-based case-control study (n=400) including a questionnaire and a biochemistry part. Biosamples are taken from patients undergoing a mastectomy (n=100 cases) or a breast reduction surgery (n=100 controls) and will be analysed in two independent laboratories (Keele and Innsbruck). The tissue is sampled respectively directly at the operating theatre during the breast reduction surgery or at the macro-diagnosis of the mastectomy supplement. The tissue is taken from three spots alongside the transect from the upper outer quadrant (axilla) to the upper inner quadrant (medial). The distances between sample spots and distances to the tumour are documented. Additionally urine and serum samples are collected from both groups. To date, we have collected biosamples from 60 interviewed women. In summary a set of 1000 biospecimen will be analysed by graphite furnace atomic absorption spectrometry.

Preliminary results and status of the study: The use of antiperspirants containing aluminium-salts and its relation to breast cancer

Caroline Linhart[1], Johanna Kowalski[2], Evi Morandi[3], Herbert Lindner[4], Heribert Talasz[4], Michael Hubalek[2], Christopher Exley[5], Nicole Concin[2] and Hanno Ulmer[1]

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The aetiology of breast cancer is likely multifactorial and involves genetic and environmental factors. Previous epidemiologic studies investigating the relationship between breast cancer and underarm antiperspirant use showed conflicting results. Recent publications suggest that breast cancer is linked to the use of antiperspirants containing aluminium-salts, which are associated with oxidative stress, proliferation and DNA double-strand breaks. We designed a hospital-based case-control study including a questionnaire and a biochemistry part. The antiperspirant use, concentration of aluminium in biosamples (breast tissue, serum, urine) and clinical/life-style data are compared between a group of 200 women suffering from breast cancer and 200 age-matched controls without breast cancer. Biosamples are taken from patients undergoing a mastectomy (cases) or a mamma reduction surgery (controls). To date, we have collected questionnaires from 220 women and biosamples from 60 women. We present challenges and lessons learned from study implementation as well as preliminary results from the questionnaire part.

PLATFORM 23

Breast cancer and the use of underarm hygiene products with aluminium-salts: A case control study.

Caroline Linhart[1], Heribert Talasz[2], Evi Morandi[3], Herbert Lindner[2], Christopher Exley[4], Nicole Conci5[2], Hanno Ulmer[1]

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[3]Department of Plastic, Reconstructive & Aesthetic Surgery, Medical University of Innsbruck, Austria
[4]The Birchall Centre, Lennard-Jones Laboratories, Keele University, UK
[5]Department of Obstetrics and Gynaecology, Medical University of Innsbruck, Austria

Previous epidemiologic studies concerning breast cancer and antiperspirant use have shown conflicting results. Therefore, we designed a hospital based case-control study including interview data and bio-samples.
Antiperspirant use and clinical records were compared between 209 women suffering from breast cancer and 209 age-matched healthy controls. Aluminium concentrations in bio-samples were measured in a subgroup of 100 cases and 52 controls.
Case-control comparisons confirmed established risk factors for breast cancer such as positive family anamnesis. A univariate significant relationship between antiperspirant application and breast cancer was identified for an intensive use under the age of 30, doubling the risk for breast cancer (p=0.045). Median (interquartile) aluminium concentration in breast tissue was 5.8 nmol/g (2.3-13.1) in cases and was significantly lower in controls (3.8 nmol/g, 2.5-5.9, p=0.0013).
First results show some indications of differences in self-reported underarm cosmetic application and aluminium concentration of tissue samples between cases and controls. These results are preliminary and await thorough multivariate analysis.

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We like to thank all medical students involved in the project: Johanna Kowalski, Silke Regensburger, Dominik Panosch, Carolin Buddensick, and Franziska Weidenbeck. Special thanks also to the secretary of the breast ambulance, Alfred Wieser.

R code for graphs and descriptive statistics

```
ALUMINUM AND BREAST CANCER: Graphs

================================================================================================
### Author: Caroline Linhart ###
================================================================================================
# Packages & Working direction #
library("PMCMR", lib.loc="~/R/win-library/3.2")
library(Hmisc)
library(psych)
library(pastecs)
# setwd #
#PC
setwd("Z:/Alu_PhD/Graphs")
#Mac
#setwd("/Volumes/NO NAME/Alu_PhD/Graphs")

#Descriptive Statistics #
f<-read.csv("Z:/Alu_PhD/Graphs/Graphs_now.csv", sep=";", dec=",")
#corr<-read.csv("Z:/Alu_PhD/Graphs/corr.csv", sep=";", dec=",")
#gradient<-read.csv("Z:/Alu_PhD/Graphs/Gradient.csv", sep=";", dec=",")
#mac
#f<-read.csv(file.choose(),sep=";",dec=",")
dim(f)
summary(f)
attach(f)
describeBy(f$Al_urine,f$LOK_AGG)
describeBy(f$Al_blood,f$LOK_AGG)
describeBy(f$MEAN_breast,f$LOK_AGG)
detach(f)
#rm(list = ls())
#dim(gradient)
#summary(gradient)

# ORDER #
summary(case$DeoUSE_NOW)
summary(control$DeoUSE_NOW)
names(f)
f$UCP_U30<-ordered(f$UCP_U30,levels=c("never","several times per week","daily","several times
per day"))
f$UCP_NOW<-ordered(f$UCP_NOW,levels=c("never","several times per week","daily","several times
per day"))
f$sport_NOW<-as.factor(f$sport_NOW)
f$Shaving_and_deo<-as.factor(f$Shaving_and_deo)
summary(f)

#subset case - control
case<-subset(f,sd_group=="case")
control<-subset(f,sd_group=="control")
summary(control)

#subset tumor LOK
UpO<-subset(f,LOK_AGG=="upper outer")
other<-subset(f,LOK_AGG=="other quadrants")
nT<-subset(f,LOK_AGG=="no tumour")

#subset tumor LOK
Up <- subset(f, LOK_AGG != "other quadrants")
table(Up$LOK_AGG)
table(droplevels(Up)$LOK_AGG)
(dim(Up))
Up$LOK_AGG<-droplevels(Up$LOK_AGG)
summary(Up)
O <- subset(f, LOK_AGG != "upper outer")
table(O$LOK_AGG)
table(droplevels(O)$LOK_AGG)
dim(O)
O$LOK_AGG<-droplevels(O$LOK_AGG)
summary(O)
```

```
#BOXPLOTS TISSUE Case - Control: Fig.9b-c

tiff("Fig9b.tiff",width = 6, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 3, 1),mgp=c(3.5,1,0))
boxplot(MEAN_breast~sd_group,
        data=f,main="Aluminium concentration of breast tissue",
        ylab="Al (nmol/g dry weight)",
        names=c("case ", "control"),
        col=c("hotpink", "palegreen"),
        cex.axis=1.5,cex.lab=1.5,
        ylim=c(0,200))
text(1.5,200,labels ="**", cex=2.2)
text(0.5,200,labels="B",cex=2)
dev.off()
```

```
tiff("Fig9d.tiff",width = 6, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 3, 1), mgp=c(3.5,1,0),par("usr")[1]+0)
boxplot(log10(MEAN_breast+1)~sd_group,
        data=f,main="Aluminium concentration of breast tissue",
        ylab="log10(Al+1) (nmol/g dry weight)",
        names=c("case ", "control"),
        col=c("hotpink", "palegreen"),
        cex.axis=1.5,cex.lab=1.5,
        ylim=c(0,2.5))
text(1.5,2.5,labels ="**", cex=2.2)
text(0.5,2.5,labels="D",cex=2)
dev.off()
```

```
Barcc<-read.csv("Z:/Alu_PhD/Graphs/Barplo_tissue_case_control.csv", sep=";", dec=",")
Barcc1<-Barcc[1:2,2:6]
BarccM<-Barcc[3:4,2:6]
tiff("Fig9c.tiff",width = 6, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 4, 1), mgp=c(3.5,1.5,0))
BarpccMc <- barplot(height = BarccM$mean,width = 1,
                    beside = TRUE, las = 1.5,
                    cex.axis=1.5,cex.lab=1.5,
                    ylim = c(0, 20),
                    cex.names = 1.5,
                    ylab = "Al (nmol/g dry weight)",
                    xlab = "Mean + 2 SE",
                    main= "Aluminium concentration in breast tissue samples",
                    names.arg=c("case","control"),
                    col=c("hotpink", "palegreen"),
                    border = "black", axes = TRUE,
                    space=1.5)
segments(BarpccMc, BarccM$mean - 0, BarpccMc,
         BarccM$mean+ BarccM$se * 2, lwd = 1.5)
arrows(BarpccMc, BarccM$mean - 0, BarpccMc,
       BarccM$mean + BarccM$se * 2, lwd = 1.5, angle = 90,
       code = 2, length = 0.05)
text(3.3,18,labels ="**", cex=2.2)
text(1.5,19,labels="C",cex=2)
box()
dev.off()
```

BOXPLOT DEO USE TISSUE Fig.10a-b

```
tiff("Fig10a.tiff",width = 7, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 3, 2), mgp=c(4,1,0),par("usr")[1]+0)
boxplot(log10(MEAN_breast+1)~UCP_NOW, data=Up,
       boxwex = 0.25, at = 1:4 - 0.15,
       subset = Up$sd_group == "case", col = ("hotpink2"),
       main = "Tumour located in the upper outer quadrant",
       xlab = "UCP use during the last 5 years",
       cex.main=1.7,
       ylab = "log10(Al+1) (nmol/g dry weight)",
       xaxt="n",
       cex.axis=1.7,cex.lab=1.7,las=1,
       xlim=c(0.75,4.25),
       ylim=c(0,2))
   axis(1,at=1:4,labels=c("never",            "several            times/week","daily","several
times/day"),cex.axis=1.7)
      boxplot(log10(MEAN_breast+1)~UCP_NOW, data=Up,add=TRUE,
        boxwex = 0.25,at=1:4 + 0.15,
        subset = Up$sd_group == "control",col=("palegreen"),
        axes=FALSE)
text(0.8,2,labels="A",cex=2)
dev.off()
```

```
tiff("Fig10b.tif",width = 7, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 3, 2), mgp=c(4,1,0),par("usr")[1]+0)
boxplot(log10(MEAN_breast+1)~UCP_NOW, data=O,
       boxwex = 0.25, at = 1:4 - 0.15,
       subset = O$sd_group == "case", col = ("hotpink2"),
       main = "Tumour located in inner or lower quadrants",
       cex.main=1.7,
       xlab = "UCP use during the last 5 years",
       ylab = "log10(Al+1) (nmol/g dry weight)",
       xaxt="n",
       cex.axis=1.7,cex.lab=1.7,las=1,
       xlim=c(0.75,4.25),
       ylim=c(0,2))
  legend(3.539,2.08, c("Case","Control"), fill=c("hotpink2","palegreen"),horiz=FALSE, cex=1.5)
  axis(1,at=1:4,labels=c("never",       "several       times/week","daily","several       times
day"),cex.axis=1.7)
boxplot(log10(MEAN_breast+1)~UCP_NOW, data=O,add=TRUE,
        boxwex = 0.25,at=1:4 + 0.15,
        subset = O$sd_group == "control",col=("palegreen"),
        axes=FALSE)
text(0.8,2,labels="B",cex=2)
dev.off()
```

```
#BARPLOT: UCP use and age of BC diagnosis: Fig.11

Bar<-read.csv("Z:/Alu_PhD/Graphs/Barplot_AgeD_UCPu30.csv", sep=";", dec=",")
Bar$UCP_useU30<-ordered(Bar$UCP_useU30,levels=c("several           times/day","daily","several
times/week","several times/month","never"))
Barh<-tapply(Bar$Mean, list(Bar$UCP_useU30),
       function(x) c(x = x))
Bar_Means <- Bar$Mean
Bar_SD <- Bar$sd
Bar_SE <- Bar$se
tiff("Fig11.tiff",width = 11.4, height = 9.4, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(10, 10, 0, 2), mgp=c(7,1,0),par("usr")[1]+0)
barpl<- barplot(Bar_Means,
                beside = TRUE, las = 1.5,
                cex.axis=2.3,cex.lab=2.5,
                ylim = c(0, 99),
                cex.names = 2,
                ylab = "Mean Age of BC Diagnosis",
                xlab = "UCP use under the age of 30",
                yaxp=c(0, max(85),17),
                space=0.4)
segments(barpl, Bar_Means - 0, barpl,Bar_Means + Bar_SD * 2, lwd = 1.5)
arrows(barpl, Bar_Means - 0, barpl, Bar_Means + Bar_SD * 2, lwd = 1.5, angle = 90,code = 2,
length = 0.05)
text(seq(0.3,6.4,by=1.4), par("usr")[3]-5,
     srt = 0, adj= 0, xpd = TRUE, labels =c("several times \n      per day","","","",""),
cex=2.2)
text(seq(0.5,6.4,by=1.4), par("usr")[3]-5,srt = 0, adj= 0, xpd = TRUE, labels =c("","","2-6
times\nper week","1-4 times\nper month",""), cex=2.2)
text(seq(0.7,6.4,by=1.4), par("usr")[3]-5,
     srt = 0, adj= 0, xpd = TRUE, labels =c("","daily","","","never"), cex=2.2)
text(seq(0.7,6.4,by=1.4), par("usr")[3]+40,
     srt = 0, adj= 0, xpd = TRUE, labels =c("42.1","","","",""), cex=2.2)
text(seq(0.7,6.4,by=1.4), par("usr")[3]+44,
     srt = 0, adj= 0, xpd = TRUE, labels =c("","45.8","","",""), cex=2.2)
text(seq(0.7,6.4,by=1.4), par("usr")[3]+47,
     srt = 0, adj= 0, xpd = TRUE, labels =c("","","49.1","",""), cex=2.2)
text(seq(0.7,6.4,by=1.4), par("usr")[3]+53,
     srt = 0, adj= 0, xpd = TRUE,labels =c("","","","54.7",""), cex=2.2)
text(seq(0.7,6.4,by=1.4), par("usr")[3]+59,
     srt = 0, adj= 0, xpd = TRUE,labels =c("","","","","61.1"), cex=2.2)
box()
dev.off()
```

```
BARPLOT: Blood and Urine: Fig.12

Barbl_ur2<-read.csv("Z:/Alu_PhD/Graphs/Barplot_blood_urine3_müperg.csv", sep=";", dec=",")
tapply(Barbl_ur2$mean, list(Barbl_ur2$sample.side, Barbl_ur2$group),function(x) c(x = x))
plotTop <- max(Barbl_ur2$mean) +
           Barbl_ur2[Barbl_ur2$mean == max(Barbl_ur2$mean), 6] * 3
Barbl_urMeans2 <- tapply(Barbl_ur2$mean, list(Barbl_ur2$sample.side,
                                    Barbl_ur2$group),function(x) c(x = x))
Barbl_urSE2 <- tapply(Barbl_ur2$se, list(Barbl_ur2$sample.side,
                                    Barbl_ur2$group),function(x) c(x = x))
tiff("Fig12.tiff",width = 6, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 4, 1), mgp=c(3.5,1.5,0))
barBarbl_ur2 <- barplot(height = Barbl_urMeans2,
                        beside = TRUE, las = 1.5,
                 cex.axis=1.5,cex.lab=1.5,
                        ylim = c(0, 13),
                        cex.names = 1.5,
                 names=c("Case upper ","Case other ", "Control"),main = "Blood and urine",
                        ylab = "Al concentration",
                        xlab = "",
                 col=c("#CB181D", "gold1","#CB181D", "gold1"),
                        border = "black", axes = TRUE,
                        legend.text = c("blood (µg/L)","urine (µg/g Crt)"),
                        args.legend = list(x = "topright",cex = 1.5))
segments(barBarbl_ur2, Barbl_urMeans2 - 0, barBarbl_ur2,Barbl_urMeans2 + Barbl_urSE2 * 2, lwd
= 1.5)
arrows(barBarbl_ur2, Barbl_urMeans2 - 0, barBarbl_ur2,Barbl_urMeans2 + Barbl_urSE2 * 2, lwd =
1.5, angle = 90,code = 2, length = 0.05)
box()
dev.off()
```

```
#BARPLOT TISSUE Gradient TB. Fig.13
```

```
BarTB <- Barcc[17:22,]
BarTB$sample.side<-droplevels(BarTB$sample.side)
tapply(BarTB$mean, list(BarTB$sample.side, BarTB$group),function(x) c(x = x))
BarTB_Means <- tapply(BarTB$mean, list(BarTB$sample.side,BarTB$group),function(x) c(x = x))
BarTBSE <- tapply(BarTB$se, list(BarTB$sample.side,BarTB$group),function(x) c(x = x))
tiff("Fig13.tiff",width = 6, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 4, 1), mgp=c(3.5,1.5,0))
Bar_GradTB <- barplot(height = BarTB_Means,
                        beside = TRUE, las = 1.5,
                        cex.axis=1.5,cex.lab=1.5,
                        ylim = c(0, 30),
                        cex.names = 1.5,names.arg = c('Cases',"Controls"),
                        main = "Gradient TB",
                       cex.main=1.7,
                        ylab = "Al (nmol/g dry weight)",
                        xlab = "Mean of sampling sites + 2 SE",
                        col=c("hotpink4",                                "hotpink3","lightpink1",
"palegreen4","palegreen3","darkseagreen1"),
                        border = "black", axes = TRUE)
legend(5.7,30, inset=.02, title="sample quadrant",
   c("upper                              outer","central","lower                                inner"),
fill=c("palegreen4","palegreen3","darkseagreen1"), horiz=FALSE, cex=1.5,)
legend(5.5,30, inset=.02, title="",
   c("","",""), fill=c("hotpink4", "hotpink3","lightpink1"), horiz=FALSE, cex=1.5, bty="n")
segments(Bar_GradTB, BarTB_Means - 0, Bar_GradTB,
         BarTB_Means + BarTBSE * 2, lwd = 1.5)
arrows(Bar_GradTB, BarTB_Means - 0, Bar_GradTB,
       BarTB_Means + BarTBSE * 2, lwd = 1.5, angle = 90,
       code = 2, length = 0.05)
box()
dev.off()
```

```
#BARPLOT: UCP & SHAVING in TISSUE: Fig.14a
```

```
alu_raz=matrix(c(7.95,12.27),nrow=1,ncol=2,byrow=TRUE)
dimnames(alu_raz) = list( c("Al"),c("separated", "together"))
raz_sd<-c(1.98,2.21)
tiff("Fig14a.tiff",width = 6, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 4, 1), mgp=c(3.5,1.5,0))
barp_deoraz <- barplot(height = alu_raz,
                        beside = TRUE, las = 1.5,
                        cex.axis=1.5,cex.lab=1.5,
                        ylim = c(0, 20),
                        cex.names = 1.5,
                        main = "Al in tissue",
                       cex.main=1.7,
                        ylab = "Al (nmol/g dry weight)",
                        xlab = "Mean + 2 SE",
                        col=c("lightblue","darkblue"),
                        border = "black", axes = TRUE,space=c(0,0.5))
segments(barp_deoraz, alu_raz - 0, barp_deoraz,
         alu_raz + raz_sd*2, lwd = 1.5)
arrows(barp_deoraz, alu_raz - 0, barp_deoraz,
       alu_raz + raz_sd*2, lwd = 1.5, angle = 90,
       code = 2, length = 0.05)
text(1.7,18,labels ="*", cex=2.2)
text(0.5,19,labels="A",cex=2)
box()
dev.off()
```

```
#BARPLOT UCP & SHAVING in blood: Fig.14b

deorazb<-read.csv("Z:/Alu_PhD/Graphs/mean_deoK.csv", sep=",", dec=".")
deorazb<-deorazb[13:16,]
deorazb$category<-droplevels(deorazb$category)
tapply(deorazb$mean, list(deorazb$category, deorazb$group),function(x) c(x = x))
deorazbMeans <- tapply(deorazb$mean, list(deorazb$category,deorazb$group),
        function(x) c(x = x))
deorazbSE <- tapply(deorazb$se, list(deorazb$category,deorazb$group),function(x) c(x = x))
tiff("Fig14b.tiff",width = 6, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 4, 1), mgp=c(3.5,1.5,0))
barp_deorazb <- barplot(height = deorazbMeans,
                        beside = TRUE, las = 1.5,
                        cex.axis=1.5,cex.lab=1.5,
                        ylim = c(0, 20),
                        cex.names = 1.5,
                        main = "Al in blood",
                        cex.main=1.7,
                        ylab = "Al (µg/L)",
                        xlab = "Mean + 2 SE",
                        col=c("lightpink","hotpink3","darkseagreen1","palegreen3"),
                        border = "black", axes = TRUE)
legend(3.72,20, inset=.02, title="shaving and application",
   c("separated","together"), fill=c("darkseagreen1","palegreen3"), horiz=FALSE, cex=1.5)
legend(3.72,20, inset=.02, title=" \n ",
   c("",""), fill=c("lightpink","hotpink3"), horiz=FALSE, cex=1.5, bty="n")
segments(barp_deorazb, deorazbMeans - 0, barp_deorazb,
         deorazbMeans + deorazbSE * 2, lwd = 1.5)
arrows(barp_deorazb, deorazbMeans - 0, barp_deorazb,
       deorazbMeans + deorazbSE * 2, lwd = 1.5, angle = 90,
       code = 2, length = 0.05)
text(1.9,19,labels ="*", cex=2.2)
text(5,10,labels ="n.s.", cex=2.2)
text(1,19,labels="B",cex=2)
box()
dev.off()
```

```
#BARPLOT: SPORT Fig.15

sport<-read.csv("Z:/Alu_PhD/Graphs/Sport_Agg.csv", sep=";", dec=",")
tiff("Fig15.tiff",width = 7, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 3, 2), mgp=c(4,1,0),par("usr")[1]+0)
boxplot(log10(Al+1)~LOK_T, data=sport,
       boxwex = 0.25, at =1:3 - 0.15,
       subset = Sport == "never-sometimes", col = c("white"),
       main = "Al",
       cex.main=1.7,
       xlab = "",
       ylab = "log10(Al+1) (nmol/g dry weight)",
       xaxt="n",
       cex.axis=1.7,cex.lab=1.7,las=1,
       xlim=c(0.65,3.65),
       ylim=c(0,2.5))
legend(0.53,2.6,c("never-sometimes","regular","often"),
fill=c("white","lightblue","royalblue2"),horiz=FALSE, cex=1.5)
axis(1,at=1.15:3.15,labels=c("Controls", "Cases (OQ)","Cases (UOQ)"),cex.axis=1.7,pos=-0.1)
x.label.position<-(xleft+xright)/2
boxplot(log10(Al+1)~LOK_T, data=sport,add=TRUE,
        boxwex = 0.25,at=1:3+0.15,
        subset = Sport == "regular",col = c("lightblue"),
        axes=FALSE)
boxplot(log10(Al+1)~LOK_T, data=sport,add=TRUE,
        boxwex = 0.25,at=1:3 + 0.45,
        subset = Sport == "often",col = c("royalblue2"),
        axes=FALSE)
text(0.65,2.5,labels="A",cex=2)
dev.off()
```

```
#BARPLOT: SPORT Fig.16

sp_b=matrix(c(5.25,4.33,3.29,12.27,7.48,6.22,8.66,15.6,23.94),nrow=3,ncol=3,byrow=TRUE)
dimnames(sp)    =    list(    c("No    tumour","Cases(OQ)","Cases    (UOQ)"),c("never-sometimes",
"regular","often"))
sp<-t(sp)
se<-c(0.63,0.94,0.55,3.62,2.21,2.44,2.41,4.14,15.01)
tiff("Fig16.tiff",width = 6, height = 6, units = "in", pointsize = 10,
     compression = "lzw", bg = "white", res = 300)
par(mar=c(6, 6, 4, 1), mgp=c(3.5,1.5,0))
bar_sport <- barplot(height = sp,
                        beside = TRUE, las = 1.5,
                        cex.axis=1.5,cex.lab=1.5,
                        ylim = c(0, 55),
                        cex.names = 1.5,
                        main = "Al in tissue",
                        cex.main=1.7,
                        ylab = "Al (nmol/g dry weight)",
                        xlab = "Mean + 2 SE",
                        col=c("white","lightblue","royalblue2"),
                        border = "black", axes = TRUE,space=c(0,0.5))
legend(1,50, inset=.02, title="physical exercise",
   c("never-sometimes","regular","often"),           fill=c("white","lightblue","royalblue2"),
horiz=FALSE, cex=1.5)
segments(bar_sport, sp - 0, bar_sport,
         sp + se*2, lwd = 1.5)
arrows(bar_sport, sp - 0, bar_sport,
       sp + se*2, lwd = 1.5, angle = 90,
       code = 2, length = 0.05)
text(2,18,labels ="*", cex=2.2)
text(5.5,18,labels ="n.s.", cex=1.8)
text(9,28,labels ="n.s.", cex=1.8)
text(0.6,53,labels="B",cex=2)
box()
dev.off()
```

SPSS syntax code for statistical analysis

Questionnaire part: conditional logistic regression analysis

```
* Univariate Logistic Regression Models of all risk Factors.
COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /METHOD=ENTER Age_Int
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /METHOD=ENTER FamilyHist_BC
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_BC_Nr)=Simple(1)
  /METHOD=ENTER FamilyHist_BC_Nr
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /METHOD=ENTER FamilyHist_OC
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Benign_BD)=Simple(1)
  /METHOD=ENTER Benign_BD
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /METHOD=ENTER Age_Menarche
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Menstruation_Cycle)=Simple(1)
  /METHOD=ENTER Menstruation_Cycle
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Hormonal_Contracept)=Simple(1)
  /METHOD=ENTER Hormonal_Contracept
  /PRINT=CI(95)
```

```
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Parity)=Simple(1)
  /METHOD=ENTER Parity
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /METHOD=ENTER Age_1st_birth_AGG_class
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Lactation)=Simple(1)
  /METHOD=ENTER Lactation
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /METHOD=ENTER Lactation_Months
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /METHOD=ENTER Menopausal_Status_Diag
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /METHOD=ENTER Age_Menopause
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (HRT)=Simple(1)
  /METHOD=ENTER HRT
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /METHOD=ENTER BMI_25_50
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Alcohol)=Simple(1)
  /METHOD=ENTER Alcohol
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

```
COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (Smoking)=Simple(1)
  /METHOD=ENTER Smoking
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (UCP_U30)=Simple(1)
  /METHOD=ENTER UCP_U30
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (UCP_NOW)=Simple(1)
  /METHOD=ENTER UCP_NOW
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

*Main adjusted Logistic Regression Models.

```
COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /CONTRAST (HRT)=Simple(1)
  /CONTRAST (UCP_U30)=Simple(1)
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /CONTRAST (Alcohol)=Simple(1)
  /CONTRAST (Benign_BD)=Simple(1)
  /METHOD=ENTER   Age_Int   FamilyHist_BC   FamilyHist_OC   Benign_BD   Age_Menarche
Age_1st_birth_AGG_class
    Menopausal_Status_Diag HRT BMI_25_50 Alcohol UCP_U30
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /CONTRAST (HRT)=Simple(1)
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /CONTRAST (Alcohol)=Simple(1)
  /CONTRAST (Benign_BD)=Simple(1)
  /CONTRAST (UCP_NOW)=Simple(1)
  /METHOD=ENTER   Age_Int   FamilyHist_BC   FamilyHist_OC   Benign_BD   Age_Menarche
Age_1st_birth_AGG_class
    Menopausal_Status_Diag HRT BMI_25_50 Alcohol UCP_NOW
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

*Incident-Time Effect Modification.

```
COMPUTE  Incident_Time1=DATEDIF(Interview_Date, Diagnosis_Date, "months").
VARIABLE LABELS  Incident_Time1 "Time between Diagnosis/Interview".
VARIABLE LEVEL  Incident_Time1 (SCALE).
FORMATS  Incident_Time1 (F5.0).
VARIABLE WIDTH  Incident_Time1(5).
EXECUTE.
```

```
COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /CONTRAST (HRT)=Simple(1)
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /CONTRAST (Alcohol)=Simple(1)
  /CONTRAST (Benign_BD)=Simple(1)
  /CONTRAST (UCP_U30)=Simple(1)
  /METHOD=ENTER   Age_Int   FamilyHist_BC   FamilyHist_OC   Benign_BD   Age_Menarche
Age_1st_birth_AGG_class
    Menopausal_Status_Diag  HRT  BMI_25_50  Alcohol  UCP_U30  UCP_U30  *  Incident_Time
Incident_Time
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /CONTRAST (HRT)=Simple(1)
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /CONTRAST (Alcohol)=Simple(1)
  /CONTRAST (Benign_BD)=Simple(1)
  /CONTRAST (UCP_NOW)=Simple(1)
  /METHOD=ENTER   Age_Int   FamilyHist_BC   FamilyHist_OC   Benign_BD   Age_Menarche
Age_1st_birth_AGG_class
Menopausal_Status_Diag  HRT  BMI_25_50  Alcohol  UCP_NOW  UCP_NOW  *  Incident_Time
Incident_Time
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

*Tumour Localisation Effect Modification and Subgroup analysis.
FREQUENCIES VARIABLES=LOK_Tumour_AGG
  /ORDER=ANALYSIS.
```

*Tumour Localisation Effect Modification.

```
COXREG DV
  /STATUS=Status(1)
  /CONTRAST (UCP_U30)=Simple(1)
  /CONTRAST (LOK_Tumour_AGG)=Simple(1)
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /CONTRAST (Benign_BD)=Simple(1)
  /CONTRAST (Alcohol)=Simple(1)
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /CONTRAST (HRT)=Simple(1)
  /METHOD=ENTER   Age_Int   FamilyHist_BC   FamilyHist_OC   Benign_BD   Age_Menarche
Age_1st_birth_AGG_class
    Menopausal_Status_Diag    HRT    BMI_25_50    Alcohol    LOK_Tumour_AGG*UCP_U30
LOK_Tumour_AGG UCP_U30
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

```
COXREG DV
  /STATUS=Status(1)
  /CONTRAST (UCP_NOW)=Simple(1)
  /CONTRAST (LOK_Tumour_AGG)=Simple(1)
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /CONTRAST (Benign_BD)=Simple(1)
  /CONTRAST (Alcohol)=Simple(1)
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /CONTRAST (HRT)=Simple(1)
  /METHOD=ENTER   Age_Int   FamilyHist_BC   FamilyHist_OC   Benign_BD   Age_Menarche
Age_1st_birth_AGG_class
    Menopausal_Status_Diag    HRT    BMI_25_50    Alcohol    LOK_Tumour_AGG*UCP_NOW
LOK_Tumour_AGG UCP_NOW
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

*Tumour Localisation Subgroup analysis.

```
DATASET ACTIVATE DataSet2.
SORT CASES  BY LOK_Tumour_AGG.
SPLIT FILE LAYERED BY LOK_Tumour_AGG.

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /CONTRAST (HRT)=Simple(1)
  /CONTRAST (UCP_U30)=Simple(1)
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /CONTRAST (Alcohol)=Simple(1)
  /CONTRAST (Benign_BD)=Simple(1)
  /METHOD=ENTER   Age_Int   FamilyHist_BC   FamilyHist_OC   Benign_BD   Age_Menarche
Age_1st_birth_AGG_class
    Menopausal_Status_Diag HRT BMI_25_50 Alcohol UCP_U30
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COXREG DV
  /STATUS=Status(1)
  /STRATA=Matched_Pairs_Rank
  /CONTRAST (FamilyHist_OC)=Simple(1)
  /CONTRAST (HRT)=Simple(1)
  /CONTRAST (Menopausal_Status_Diag)=Simple(1)
  /CONTRAST (Age_1st_birth_AGG_class)=Simple(1)
  /CONTRAST (FamilyHist_BC)=Simple(1)
  /CONTRAST (Alcohol)=Simple(1)
  /CONTRAST (Benign_BD)=Simple(1)
  /CONTRAST (UCP_NOW)=Simple(1)
  /METHOD=ENTER   Age_Int   FamilyHist_BC   FamilyHist_OC   Benign_BD   Age_Menarche
Age_1st_birth_AGG_class
    Menopausal_Status_Diag HRT BMI_25_50 Alcohol UCP_NOW
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

Bio-sample part: general linear models (GLMs)

```
COMPUTE MEAN_Breast=MEAN(Al_1r,Al_2r,Al_3r,Al_1l,Al_2l,Al_3l).
EXECUTE.
COMPUTE Al1=MEAN(Al_1r,Al_1l).
EXECUTE.
COMPUTE Al2=MEAN(Al_2r,Al_2l).
EXECUTE.
COMPUTE Al3=MEAN(Al_3r,Al_3l).
EXECUTE.
COMPUTE logMean_Breast=LG10(MEAN_Breast+1).
EXECUTE.
COMPUTE logAl1=LG10(Al1+1).
EXECUTE.
COMPUTE logAl2=LG10(Al2+1).
EXECUTE.
COMPUTE logAl3=LG10(Al3+1).
EXECUTE.
```

* Case-Control Comparison.

```
T-TEST GROUPS=Group(0 1)
  /MISSING=ANALYSIS
  /VARIABLES=logMean_Breast
  /CRITERIA=CI(.95).
```

* Main Model 3-way ANOVA.

```
GLM logAl1 logAl2 logAl3 BY group WITH UCP_U30_AGG
  /WSFACTOR=sampling_location 3 Polynomial
  /METHOD=SSTYPE(3)
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=sampling_location
  /DESIGN=UCP_U30_AGG Group.

GLM logAl1 logAl2 logAl3 BY group WITH UCP_NOW_AGG
  /WSFACTOR=sampling_location 3 Polynomial
  /METHOD=SSTYPE(3)
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=sampling_location
  /DESIGN=UCP_NOW_AGG Group.
```

* Interaction / Subgroup analysis regarding tumour localisation.

```
RECODE LOK_Tumour (0=0) (1=1) (2=2) (3=1) INTO LOK_Tumour_AGG.
EXECUTE.

USE ALL.
COMPUTE filter_$=(Group = 1).
VARIABLE LABELS filter_$ 'Group = 1 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

DATASET ACTIVATE DataSet1.
FREQUENCIES VARIABLES=LOK_Tumour_AGG
  /ORDER=ANALYSIS.

FILTER OFF.
USE ALL.
EXECUTE.
```

* Cases with tumours in upper outer quadrants and controls.

```
USE ALL.
COMPUTE filter_$=(LOK_Tumour_AGG = 0  | LOK_Tumour_AGG =2).
VARIABLE LABELS filter_$ 'LOK_Tumour_AGG = 0  | LOK_Tumour_AGG =2 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.
```

```
* Case-Control Comparison.
T-TEST GROUPS=Group(0 1)
  /MISSING=ANALYSIS
  /VARIABLES=logMean_Breast
  /CRITERIA=CI(.95).

GLM logAl1 logAl2 logAl3 BY group WITH UCP_U30_AGG
  /WSFACTOR=sampling_location 3 Polynomial
  /METHOD=SSTYPE(3)
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=sampling_location
  /DESIGN=UCP_U30_AGG Group.

GLM logAl1 logAl2 logAl3 BY group WITH UCP_NOW_AGG
  /WSFACTOR=sampling_location 3 Polynomial
  /METHOD=SSTYPE(3)
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=sampling_location
  /DESIGN=UCP_NOW_AGG Group.

FILTER OFF.
USE ALL.
EXECUTE.

* Cases with tumours in other quadrants and controls.
USE ALL.
COMPUTE filter_$=(LOK_Tumour_AGG = 0  | LOK_Tumour_AGG =1).
VARIABLE LABELS filter_$ 'LOK_Tumour_AGG = 0  | LOK_Tumour_AGG =1 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

* Custom Tables.
CTABLES
  /VLABELS VARIABLES=MEAN_Breast UCP_U30_AGG UCP_NOW_AGG Group DISPLAY=LABEL
  /TABLE MEAN_Breast [MEAN, STDDEV, MEDIAN, PTILE 25, PTILE 75, VALIDN F40.0] +
UCP_U30_AGG [C] >
    MEAN_Breast [S][MEAN, STDDEV, MEDIAN, PTILE 25, PTILE 75, VALIDN F40.0] +
UCP_NOW_AGG [C] >
    MEAN_Breast [S][MEAN, STDDEV, MEDIAN, PTILE 25, PTILE 75, VALIDN F40.0] BY
Group [C]
  /CATEGORIES VARIABLES=UCP_U30_AGG UCP_NOW_AGG ORDER=A KEY=VALUE EMPTY=INCLUDE
  /CATEGORIES VARIABLES=Group [1, 0, OTHERNM] EMPTY=INCLUDE.

* Case-Control Comparison.
T-TEST GROUPS=Group(0 1)
  /MISSING=ANALYSIS
  /VARIABLES=logMean_Breast
  /CRITERIA=CI(.95).

GLM logAl1 logAl2 logAl3 BY group WITH UCP_U30_AGG
  /WSFACTOR=sampling_location 3 Polynomial
  /METHOD=SSTYPE(3)
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=sampling_location
  /DESIGN=UCP_U30_AGG Group.

GLM logAl1 logAl2 logAl3 BY group WITH UCP_NOW_AGG
  /WSFACTOR=sampling_location 3 Polynomial
  /METHOD=SSTYPE(3)
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=sampling_location
  /DESIGN=UCP_NOW_AGG Group.

FILTER OFF.
USE ALL.
EXECUTE.
```

```
GLM logAl1 logAl2 logAl3 BY LOK_Tumour_AGG Group WITH UCP_NOW_agg
  /WSFACTOR=sampling_location 3 Polynomial
  /METHOD=SSTYPE(3)
  /PRINT=DESCRIPTIVE
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=sampling_location
  /DESIGN=UCP_NOW_agg LOK_Tumour_AGG(Group).

GLM logAl1 logAl2 logAl3 BY LOK_Tumour_AGG Group WITH UCP_U30_agg
  /WSFACTOR=sampling_location 3 Polynomial
  /METHOD=SSTYPE(3)
  /PRINT=DESCRIPTIVE
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=sampling_location
  /DESIGN=UCP_U30_agg LOK_Tumour_AGG(Group).
```

Caroline Linhart – List of publications

[1] Desole S, Watzinger K, **Linhart C**, Kähler C. Die Aktivierung von Notch-Rezeptoren stimuliert die Migration von humanen neutrophilen Granulozyten. *Pneumologie* 2012; 66: P11.

[2] Zembacher R, Desole S, **Linhart C**, Watzinger K, Kähler C. Jagged-1 stimuliert die Proliferation von Alveolar Typ II Zellen der Ratte in vitro. *Pneumologie* 2012; 66: P18.

[3] Gaughan J, Kobel C, **Linhart C**, Mason A, Street A, Ward P. Why do patients having coronary artery bypass grafts have different costs or length of stay? An analysis across 10 European countries. *Heal Econ (United Kingdom)* 2012; 21: 77–88.

[4] **Linhart C**, Schagerl M. Seasonal succession of the travertine-forming desmid Oocardium stratum. *J Phycol* 2015; 51: 1055–65.

[5] Draper N, Giles D, Schöffl V, Konstantin Fuss F, Watts P, Wolf P, **Linhart C**, et al. Comparative grading scales, statistical analyses, climber descriptors and ability grouping: International Rock Climbing Research Association position statement. *Sport Technol* 2015; 8: 88–94.

[6] **Linhart C**, Talasz H, Morandi EM, Exley C, Lindner HH, Taucher S, et al. Use of Underarm Cosmetic Products in Relation to Risk of Breast Cancer: A Case-Control Study. *EBioMedicine* 2017; 21: 79–85.

[7] Oberacher H, Arnhard K, **Linhart C**, Diwo A, Marksteiner J, Humpel C. Targeted Metabolomic Analysis of Soluble Lysates from Platelets of Patients with Mild Cognitive Impairment and Alzheimer's Disease Compared to Healthy Controls: Is PC aeC40:4 a Promising Diagnostic Tool? *J Alzheimer's Dis* 2017; 57: 493–504.

[8] Zehetner C,, Moelgg M, **Linhart C**, Bechrakis NE. In vitro flow analysis of novel double-cutting, open-port, ultrahigh-speed vitrectomy systems. *Retin J Retin Vitr Dis* 2017. (accepted Aug 27, 2017)

[9] Braito M, Schlager A, Wansch J, **Linhart C**, Biederman R. Continuous Wound Infiltration after Hallux Valgus Surgery A prospective, randomized, double-blind and placebo-controlled single-center trial. *Foot Ankle Int* 2017. (accepted Sep 09, 2017)

[10] Jones K, **Linhart C**, Hawkins C, Exley C. Urinary excretion of aluminium and silicon in secondary progressive multiple sclerosis. Submitted to *EBioMedicine* 21.9.2017

Caroline Linhart – Contribution to conferences

[1] **Linhart C**, Talasz H, Morandi EM, Exley C, Lindner HH, Concin N, Ulmer H. Breast cancer and the use of underarm hygiene products with aluminium-salts: A case-control study. Oral presentation at the 12th Keele Meeting on Aluminium – Living in the Aluminium Age, Vancouver, 2017.

[2] **Linhart C**, Talasz H, Morandi EM, Exley C, Lindner HH, , Concin N, Ulmer H. Brust – Antiperspirant aluminium salts and breast cancer: Preliminary data from a case control study. Oral presentation at the 56. Wissenschaftliche Jahrestagung der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V., München, 2016.

[3] Panosch D, Weidenbeck F, Hubalek M, Morandi E, Lindner HH, Talasz H, Exley C, Concin N, Ulmer H, **Linhart C**. The use of antiperspirants containing aluminium-salts and its relation to breast cancer: Methods and implementation of bio-specimen sampling. Poster at the 11th Keele Meeting on Aluminium – The Natural History of Aluminium. Past, Present and Future, Lille, 2015.

[4] **Linhart C**, Kowalski J, Morandi EM, Lindner HH, Talasz H, et al. Hubalek M, Exley C, Concin N, Ulmer H. Preliminary results and status of the study: The use of antiperspirants containing aluminium-salts and its relation to breast cancer. Oral presentation and poster at the 11th Keele Meeting on Aluminium – The Natural History of Aluminium. Past, Present and Future, Lille, 2015.

[5] **Linhart C.** Aluminiumsalze als Biomarker bei der Ätiologie des Mammakarzinoms. Beispiel eines Studiendesigns für die Etablierung von Biomarkern. Oral presentation at the Annual Symposium of the Austrian Society for Quality Insurance and Standardisation for medical and diagnostic Analysis (ÖQUASTA), Igls, Sept. 2013.

[6] **Linhart C**, Concin N, Kowalski J, Morandi EM, Exley C, Lindner HH, Talasz H, Ulmer H. The use of antiperspirants with aluminium salts and its relation to breast cancer. Poster presentation at the meeting of the International Biometric Society (IBS) Austro-Swiss Region (ROeS), Dornbirn, Sept. 2013.

[7] **Linhart C**, Concin N, Taucher S, Lindner HH, Ulmer H. Antiperspirants with aluminium salts and the relation to breast cancer. Poster presentation at the 10th Keele Meeting on Aluminium, Winchester, 2013.

Caroline Linhart – Curriculum vitae

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Personal data

Date of birth and place	May 22nd 1985, Scheibbs, lower Austria
Nationality	Austria

Education

2014-10/2017	**PhD Student, Medical University Innsbruck** Program: Genetics and Genomics PhD Project: *'The use of underarm cosmetic products in relation to breast cancer. A case-control study'*
2013-8/2017	**University Assistant & PhD Student** Medical University Innsbruck
11/2010-12/2012	**Euro-DRG Project collaborator** Medical University Innsbruck
09/2004-07/2011	**University of Vienna** Main subject: aquatic ecology
19/07/2011	2nd diploma part graduation with excellence: aquatic microbiology and limnology
01/2010 – 07/2010	Exchange term at **NTNU Trondheim**
03/2008	Start of the master thesis (diploma thesis): *'Autecology of Oocardium stratum and $CaCO_3$ precipitation of autotrophic biofilms in travertine rivulets'* Supervisor: Prof. Mag. Dr. M. Schagerl
03/2006	1st diploma examination: Biology
09/2004 – 07/2011	Study of biology at the University of Vienna and Kulturtechnik und Wasserwirtschaft **BOKU**
09/2003 – 09/2004	Biotechnology FH Campus Vienna
	School education
09/1999 – 06/2003	Grammar school: BORG Scheibbs, Lower Austria

Work experience

08/2013-8/2017	**University Assistant** at the Medicine University of Innsbruck, Department of Medical Statistics, Informatics and Health Economy (MSIG): **Lecturer** for 'Statistics' and 'Medical Science' Consultant for medical statistics, study design and research questions. **PhD Thesis**: *The use of antiperspirants containing Aluminium salts and its relation to breast cancer*. Focus on Statistics (R), programming, analysing of bio-samples with GF-AAS and molecular impacts of aluminium salts.
11/2012 –08/2013	**Free scientific collaborator at the University of Innsbruck**, Department of ecology: field work and statistics (Bacteria abundance and fatty acid amount in a glacial habitat: climate change factors)
	Free Scientific associate at the Medicine University of Innsbruck, Department of Medical Statistics, Informatics and Health Economy (MSIG): Focus on Statistics (R), programming, writing and submission of applications for project funding. (ecotoxicology of aluminium and breast cancer)
02/2012 – 09/2012	**Scientific associate at the Medicine University of Innsbruck** Department of Inner Medicine, Lab of Inflammation.
11/2010 – 01/2012	**Scientific associate at the Medicine University of Innsbruck** Department of Medical Statistics, Informatics and Health Economy (MSIG). Focus on Statistics (R) and programming for the **EuroDRG** project.
2009	**Wasserkluster Lunz**, contract for services, identification of freshwater algae, microscopy and field work.
2009	**Umweltbüro Blattfisch** minor employment: Macrozoobenthos and field work http://www.blattfisch.at/158.0.html
05 – 08/2007	**Niederösterreichische Umweltanalytik (NUA)**, minor employment: field work, sampling, water chemistry
03/2006	**eb&p Umweltbüro Klagenfurt**, summer internship, field work, identification of water side vegetation
08/2004	**Baxter Bioscience**, summer internship, lab work
1999 – 2003	**Biologische Station Lunz/See NÖ**, respectively 3-4 weeks, summer internship, field work.

Scientific qualification

Analytical techniques / microscopy	GF-ASS Water chemistry Analysis, Titrations High performance Liquid Chromatography (HPLC) DAPI-staining Epifluorescence Microscopy Measurements of Fluorescent with Pulse Amplitude Modulation (PAM) PCR, Boyden Chamber technique
Programming, statistics and computer skills	R, SPSS, STATA, Sigma Plot, MS-Office, EZ-Chrom (HPLC), Chemtax Endnote, Papers, Mendeley, Adobe Photoshop, ArcGIS, Geomedia

Languages

German	Mother tongue
English	Excellent
French	Intermediate
Norwegian	Beginner

Additional employments

2002-2009	Active Member of the Austrian Mountain Rescue Service, (Lackenhof/Ötscher)
2003-2008	Ski- and Snowboardinstructor

Additional skills

2009	Climbing Instructor of the ÖAV, Austrian Alpine Club
2008	Padi Open Water Diving License
2005	Ski- and Snowboardinstructor

Congress participations

2017	**12h Anniversary Keele Meeting on Aluminium**, 23rd- 27th February 2017,Vancouver, Canada. Nomination for a postgraduate bursary. 20 min platform presentation, invited speaker. http://www.keele.ac.uk/aluminium/keelemeetings/2017/
2016	**DGAUM: 56th Wissenschaftliche Jahrestagung der deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin**. 9th-11th March 2016, Munich, Germany. Invited speaker. https://www.dgaum.de/dgaum-jahrestagung/archiv-jahrestagungen/jahrestagung-2016/
2015	**11th Anniversary Keele Meeting on Aluminium**, 23rd- 27th February 2015, Lille, France Nomination for a postgraduate bursary. 20 min platform presentation http://www.keele.ac.uk/aluminium/keelemeetings/2015/

2014	**15th BfR-Forum Verbraucherschutz** **Aluminium im Alltag: Ein gesundheitliches Risiko.** 26th-27th Nov. 2014, Berlin
2013	**10th Anniversary Keele Meeting on Aluminium**, 23rd- 27th February 2013, Winchester, UK. Nomination for a postgraduate bursary. Poster presentation http://www.keele.ac.uk/aluminium/keelemeetings/2013/
2012	**ERS, European Respiratory Society, Congress,** 1st – 5th September 2012, Vienna, Austria. http://www.erscongress2012.org/
2011	**EuroDRG: final conference,** 16th-17th November 2011, Berlin, Germany. http://eurodrg.projects.tu-berlin.de/
2008	**Fresh Blood for Fresh Water,** 16th -18th May 2008, Lunz am See, Austria. Young Aquatic Science meeting. Poster presentation

Innsbruck, am 30.7.2017

Mag. Caroline Linhart