Predictors of Lymph Node Metastasis in Primary Breast Cancer - Risk Models for Tailored Axillary Management

Dihge, Looket

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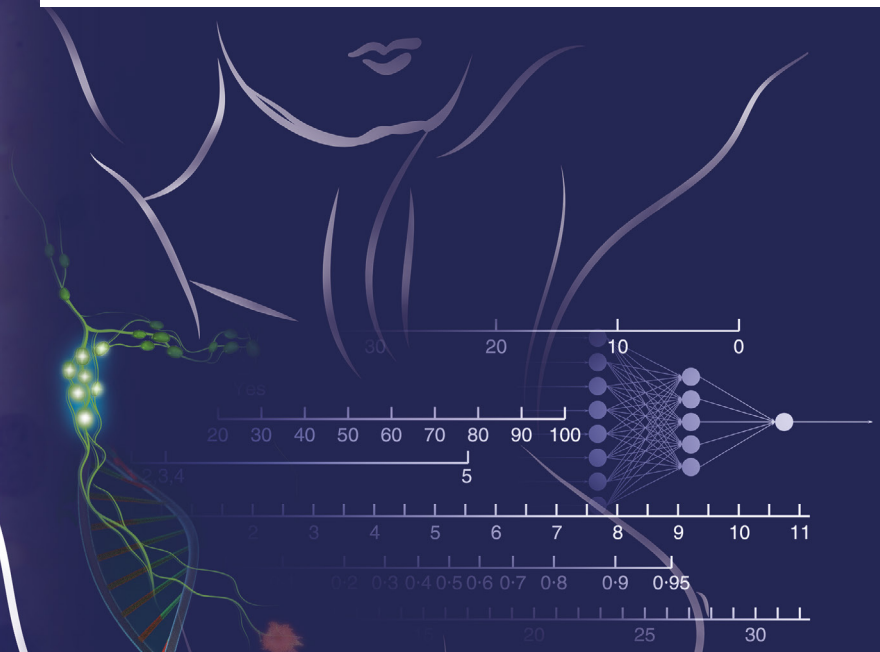
Risk Models for Tailored Axillary Management

LOOKET DIHGE
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY



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Looket Dihge

LUND
UNIVERSITY

DOCTORAL DISSERTATION
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Faculty opponent
Professor Thorsten Kuehn, M.D., Ph.D.
Teaching Hospital for Eberhard Karls-Universität, Esslingen, Germany

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Predictors of Lymph Node Metastasis in Primary Breast Cancer - Risk Models for Tailored Axillary Management

Most patients with breast cancer present with low-risk tumors, node-negative disease, and excellent prognosis. For these patients, routine axillary nodal staging by sentinel lymph node biopsy (SLNB) has no therapeutic benefit. For patients with limited sentinel lymph node metastasis, completion axillary nodal dissection is controversial. Furthermore, those with heavy-burden metastasis could benefit from preoperative selection for neoadjuvant treatment and/or more extensive axillary nodal excision rather than SLNB.

This thesis present results on the utility of axillary ultrasonography (AUS), as well as novel prediction models for the estimating disease-free axilla, limited axillary nodal metastasis, and heavy-burden axillary nodal metastasis.

Study I The sensitivity of AUS to detect metastatic nodal disease was low with a high false negative rate. Axillary metastatic burden, defined by metastatic size and number of involved nodes, was the most important predictor of an abnormal AUS. This suggest that AUS is unreliable in patients with low metastatic burden. Histological grade was found to be an independent factor that effected the accuracy of AUS performance. Patients with HER2-positive tumors were found to have higher rates of AUS abnormalities. The overall axillary metastatic burden was higher in patients with preoperative verified nodal metastasis by AUS-guided biopsy compared with those with normal AUS findings but with metastatic sentinel lymph node.

Study II Breast cancer surrogate molecular subtypes, age, mode of detection, tumor size, multifocality, and vascular invasion were identified as predictors of nodal disease in patients with T1-T2 breast cancer. Three nomograms that included these predictors were developed to predict disease-free axilla N0, limited axillary nodal metastasis (1-2 positive lymph nodes), and heavy-burden axillary nodal metastasis (≥ 3 positive lymph nodes). Area under the ROC curves (AUCs) ranged from 0.70–0.81. The increase in tumor size was found to be less often associated with metastatic nodal involvement in the TNBC subtype than in other non-TNBC subtypes.

Study III Clinicopathological characteristics were incorporated into artificial neural network models to predict disease-free axilla N0, low-burden metastasis (1-3 positive nodes), and heavy-burden metastasis (≥ 4 positive nodes) in patients with clinically node-negative breast cancer. Tumor size, LVI, and multifocality displayed linear correlation patterns to the nodal status end-points, while other predictors (age, histological type, ER status, PR status, Ki-67 values, mode of detection, and tumor localization in the breast) revealed non-linear dynamic associations. The clinical utility of reducing unnecessary SLNB was assessed; a cut-off value according to maximum negative predictive value or false-negative rate of 5–10% in a model to discriminate disease-free axilla yielded a SLNB reduction rate of 8–27%.

Study IV Predictors of nodal metastasis were assessed using clinicopathological characteristics, gene expression data, and combined features. In the overall validation cohort, the predictor with combined features showed the highest discriminative performance (AUC 0.72). However, discriminatory performances were highly similar using clinicopathological predictors alone across the surrogate molecular subtypes based on the ER, PR, and HER2 status. Higher proportions of the luminal B intrinsic features and proliferation-related genes were observed in predicted node-positive ER+HER2- and HER2+ tumors, while low-expression of basal-like markers were observed in predicted node-positive TNBC tumors.

In conclusion, these studies demonstrate the ability to estimate axillary nodal burden using preoperatively obtainable predictors and highlight nonlinear associations between clinicopathological variables and nodal metastasis. Preoperative prediction of the nodal status would facilitate individualized axillary management.

Key words: Breast cancer, axilla, lymph node excision, ultrasonography, nomogram, gene expression

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Looket Dihge, M.D.

Faculty of Medicine
Division of Surgery
Department of Clinical Sciences, Lund

LUND
UNIVERSITY

Supervisor: Professor Lisa Rydén, M.D., Ph.D.
Faculty of Medicine
Division of Surgery
Department of Clinical Sciences, Lund

Co-supervisor: Associate Professor Pär-Ola Bendahl, Ph.D.
Faculty of Medicine
Division of Oncology and Pathology
Department of Clinical Sciences, Lund

Co-supervisor: Associate Professor Johan Staaf, Ph.D.
Faculty of Medicine
Division of Oncology and Pathology
Department of Clinical Sciences, Lund

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Faculty of Medicine
Department of Clinical Sciences Lund

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To Aree,
Lars and Paibulya

"Medicine is not only a science; it is also an art.
It does not consist of compounding pills and plasters;
it deals with the very processes of life,
which must be understood before they may be guided."

– Paracelsus, 1493-1541

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Summary in Swedish - Populärvetenskaplig sammanfattning

1,7 miljoner nya fall av bröstcancer diagnosticeras årligen i världen. Bröstcancer är kvinnans vanligaste tumörsjukdom och i västvärlden samt Sverige kommer var åttonde till var nionde kvinna att insjukna under sin livstid. Överlevnaden efter bröstcancer är bland de högsta i världen i Sverige, med femårsöverlevnad på cirka 90%. För de kvinnor som diagnosticeras i ett tidigt stadium är femårsöverlevnaden närmare 100%, vilket återspeglar att överlevnaden påverkas allra starkast av om cancersjukdomen spridit sig utanför bröstet eller ej.

Spridning av bröstcancerceller från brösttumören sker genom lymfflödet till lymfkörtlarna i armhålan (axillen) på samma sida som det cancerdrabbade bröstet. Kunskap om spridning av bröstcancerceller till lymfkörtlarna, lymfkörtelmetastaser, ger värdefull information om sjukdomens förlopp, och är viktigt för valet av återfallsförebyggande behandling. Kirurgisk avlägsnande av tumören i bröstet utgör grunden för bröstcancerbehandlingen och samtidigt görs även rutinmässigt en operation i armhålan för uttag av lymfkörtlar för att verifiera möjlig förekomst av lymfkörtelmetastaser. Portvaktskörtlar kallas de lymfkörtlar som först tar emot lymfvätskan från bröstet, och portvaktskörtelkirurgi är metoden för att identifiera och avlägsna portvaktskörtlarna för analys. I jämförelse med axillutrymning, som är ett mer omfattande kirurgiskt ingrepp där fler lymfkörtlar, lymfatisk vävnad och fett avlägsnas, minskar portvaktskirurgi patientens risk att få påverkan av armrörlighet, armsvullnad och smärttillstånd som biverkningar av lymfkörteloperationen.

Majoriteten av bröstcancerpatienterna diagnostiseras i ett tidigt sjukdomsskede, och allt färre har lymfkörtelmetastaser. Portvaktskörtelkirurgen påvisar idag friska portvaktskörtlar hos 70-85% av patienterna. För dessa saknar det diagnostiska kirurgiska ingreppet i axillen behandlingsvärde, och operationen skulle kunna avvaras om lymfkörtelstatus kunde bedömas utan kirurgi. Användning av effektiv tilläggsbehandling efter operation bidrar till en allt bättre sjukdomskontroll. Valet av tilläggsbehandling baseras idag i stor utsträckning på tumörens biologiska egenskaper och begränsas inte längre enbart till informationen om antalet tumörbärande lymfkörtlar.

De fyra ingående studierna i avhandlingen syftar till att undersöka faktorer som är kopplade till bröstcancerspridning till lymfkörtlarna, och utreda möjligheten att utvärdera diagnostiska verktyg för att uppskatta graden av lymfkörtelspridningen. Studie I–III utgår från 1172 patienter som opererats vid Skånes universitetssjukhus i Lund under 2009-2012 till följd av bröstcancer eller cancermisstänkt bröstförändring. Studie IV utgår från 3023 patienter som diagnostiserades med bröstcancer under 2010-2015 och som har valt att delta i SCAN-B studien, där

genomiska analyser av tumörvävnaden genomförs. Information har inhämtats från patologidatabaser, patientjournaler och från nationella kvalitetsregister.

Intresset för att använda bilddiagnostisk teknik för kartläggning av axillens lymfkörtlar tilltar, men träffsäkerhet är omdebatterad. Inom bröstcancervård har ultraljud länge använts för att bedöma cancertumören i bröstet och armhålans lymfkörtlar. I studie I fann vi att tekniken även kan upptäcka om det föreligger utbredd tumörsjukdom i lymfkörtlarna. Däremot var tillförlitligheten sämre för att visualisera mindre tumörhärdar, eller säkerställa om cancerspridningen enbart inbegriper ett fåtal lymfkörtlar. Med ultraljudsbilden som vägledning kan celler från en misstänkt tumörinnehållande lymfkörtel dras ut och undersökas med biopsi. Bekräftar analysen att det föreligger spridning är den totala tumörhärden i axillens lymfkörtlar större än om spridningen skulle upptäckas vid portvaktskörtelkirurgin. Patienterna kan då ha god nytta av att inleda cellhämmande läkemedel (cytostatika) redan innan bröstcanceroperation, och av att genomgå axillutrymning direkt utan ett kirurgiskt mellansteg via portvaktskörtelkirurgi.

Resultaten från samtliga studier bekräftade att tumörens storlek vid diagnos, antalet tumörer och om cancerceller invaderat lymfkärl eller inte, är av central betydelse för risken att brösttumören skall ha spridits till lymfkörtlarna. I studie II-IV fann vi att tumörbiologiska egenskaper, såsom tumörens känslighet för kvinnliga könshormoner för tillväxt, kan påverka risken för lymfkörtelspridningen, men också omfattningen av den totala sjukdomsbördan i axillen. Faktorer som patientens ålder vid diagnos och om tumören upptäcktes via hälsokontroll med mammografi uppvisade relevans för omfattningen av lymfkörtelspridningen. I studie III noterades att även cancerns läge i bröstet och cancerns specifika vävnadstyp, om den utgår från bröstkörtelgång eller ej, kunde inverka på omfånget av lymfkörtel-spridningen i axillen.

Faktorer som är kopplade till spridningsrisken sammanställdes till tre grafiska beräkningsdiagram (nomogram) i studie II. Genom poängskalor, motsvarande exempelvis patientens ålder, aktuell tumörstorlek i mm och tumörbiologiska egenskaper, räknades en totalsumma fram. De tre modellerna ger uppskattning om patientens möjlighet att ha friska lymfkörtlar, om hennes risk att ha begränsad sjukdomsspridning till lymfkörtlarna (1-2 sjukliga lymfkörtlar) samt om risken för en mer omfattande tumörbörda. Att kunna skilja mellan olika omfattning av sjukdomsspridningen är avgörande för planering av det kirurgiska ingreppet i armhålan. Alltfler studier har påvisat att kompletterande axillutrymning kan undvaras hos patienter där cancerspridningen till portvaktskörtlar är begränsad (små tumörhärdar eller omfattar ett fåtal portvaktskörtlar). Studierna har pekat på att biverkningar från lymfkörtelkirurgin har kunnat minskas till följd av utebliven axillutrymning utan ökad risk att dö i bröstcancersjukdomen.

Faktorer som kan påverka lymfkörtelspridningen har undersökts i studie III med hjälp av artificiella neurala nätverk (ANN). ANN är datoriserade modeller som kan hantera komplexa beräkningar och finna mönster mellan olika faktorer och givet utfall. Modellerna uppvisade att komplexa samband föreligger mellan patient- och tumörfaktorer och graden av sjukdomsspridning. En faktors betydelse för lymfkörtelstatus är inte statisk, utan har visat sig påverkas av andra faktorer i svårförutsägbara sambandsmönster. Tre beräkningsmodeller skapades för att förutsäga möjligheten för sjukdomsfrihet i lymfkörtlarna, samt risken för att drabbas av begränsad eller mer omfattande cancerspridning till lymfkörtlarna. Beräkningsmodellerna visade en bättre förmåga till att kunna förutse de olika graderna av lymfkörtelsjuklighet jämfört med mer traditionella statistiska modeller. Att uppskatta möjligheten att ha friska lymfkörtlar är viktigt för att kunna identifiera patienter med mycket låg risk för lymfkörtelspridning, och därmed undvara en onödig portvaktskörtelkirurgi.

I studie IV undersöktes tumörens genetiska uttryck, kopplade till regleringen av cancercellernas delning och tumörens tillväxt, och samband med lymfkörtelspridningen. Styrkan i sambandet mellan det genetiska uttrycket varierade mellan olika typer av bröstcancertumörer, och var mest påtaglig för den tumörgrupp som är känslig för kvinnliga könshormoner för sin tillväxt, eller för tumörer med ökad mängd av äggviteämnet HER2. Trots kännedom om tumörens genetiska uttryck visade studien att tumörens storlek, och om cancercellerna har invaderat lymfkärl, är fortsatt väsentliga faktorer för att förutsäga sjukdomsspridningen. Genetiska profiler kunde inte ersätta betydelsen av tumörstorlek för att bedöma risken för lymfkörtelspridningen.

Sammantaget visar avhandlingen att patientrelaterade faktorer och tumörens biologiska egenskaper inverkar på cancerspridningen till lymfkörtlarna, och omfattningen av antalet sjuka lymfkörtlar. Diagnostiska verktyg baserade på dessa faktorer kan därför bidra till att förutsäga lymfkörtelstatus, och därmed förbättrade möjligheter till en mer individanpassad kirurgisk bröstcancerbehandling. Framförallt kan kartläggningen av lymfkörtelstatus i ett tidigt skede, med stöd av diagnostiska verktyg, bidra till att minska antalet kirurgiska ingrepp i axillen som inte medför någon behandlingsnytta för patienten.

Summary in Thai -
บทคัดย่อดุษฎีนิพนธ์ภาษาไทย

บทคัดย่อดุษฎีนิพนธ์ฉบับนี้เป็นการแปลและสรุปดุษฎีนิพนธ์เป็นภาษาไทยง่ายๆสำหรับผู้รู้ภาษาไทยทั่วๆไปอ่านแล้วพอเข้าใจได้

ในแต่ละปีมีผู้ป่วยตรวจพบมะเร็งเต้านมรายใหม่ถึง 1.7 ล้านรายทั่วโลก มะเร็งเต้านมเป็นมะเร็งที่ตรวจพบมากที่สุดในผู้หญิงทั่วโลก โดยเฉพาะในผู้หญิงจากซีกโลกตะวันตก ผู้หญิงที่ป่วยเป็นมะเร็งเต้านมในประเทศสวีเดนมีอัตราการรอดชีวิตอยู่ในกลุ่มที่สูงที่สุดในโลก โดยมีอัตราการรอดชีวิตภายในระยะเวลา 5 ปีประมาณ 90% และถ้าหากตรวจพบมะเร็งเต้านมในระยะเริ่มแรกไม่มีการแพร่กระจายจะมีอัตราการรอดชีวิตภายใน 5 ปีมากถึง 100% สะท้อนให้เห็นว่าการตรวจพบมะเร็งเต้านมก่อนที่มะเร็งจะแพร่กระจายออกไปจากเต้านมมีความสำคัญทำให้มีอัตราการรอดชีวิตสูงขึ้น

เซลล์มะเร็งเต้านมสามารถแพร่กระจายไปกับน้ำเหลืองเข้าสู่ต่อมน้ำเหลืองบริเวณรักแร้ด้านเดียวกับเต้านมที่เป็นมะเร็ง ความรู้เกี่ยวกับสถานะของต่อมน้ำเหลืองในผู้ป่วยมะเร็งเต้านมมีความสำคัญต่อการตรวจวินิจฉัยโรค วิธีการรักษาโรค และวิธีการป้องกันการลุกลามของโรคมะเร็งเต้านม

ผู้ป่วยมะเร็งเต้านมส่วนใหญ่ในปัจจุบันได้รับการวินิจฉัยเป็นมะเร็งเต้านมในระยะเริ่มแรก ฉะนั้นอัตราการแพร่กระจายของมะเร็งไปยังต่อมน้ำเหลืองในปัจจุบันลดน้อยลง ในประมาณ 70-85% ของผู้ป่วยมะเร็งเต้านมทั้งหมดแพทย์จะไม่พบการแพร่กระจายมะเร็งไปยังต่อมน้ำเหลืองบริเวณรักแร้ ถ้าผลการตรวจต่อมน้ำเหลืองกลุ่มแรกในบริเวณซอกรักแร้ที่ได้รับน้ำเหลืองจากก้อนมะเร็งในเต้านม (การตรวจต่อมน้ำเหลืองเซนติเนล Sentinel lymph node biopsy) แล้วไม่พบการแพร่กระจายของเซลล์มะเร็งแพทย์จะไม่จำเป็นต้องผ่าตัดต่อมน้ำเหลืองทั้งหมดในบริเวณรักแร้ออกไป การตรวจวิธีการแพร่กระจายไปยังต่อมน้ำเหลืองด้วยวิธีตรวจต่อมน้ำเหลืองเซนติเนลจะช่วยลดผลข้างเคียงที่เกิดจากวิธีการผ่าตัดแบบวงกว้างในบริเวณรักแร้

การศึกษาดุษฎีนิพนธ์ฉบับนี้มีวัตถุประสงค์ศึกษาปัจจัยการแพร่กระจายของโรคมะเร็งไปยังต่อมน้ำเหลือง สร้างเครื่องมือวิเคราะห์ขอบเขตของการแพร่กระจายของโรคมะเร็งไปยังต่อมน้ำเหลืองและประเมินการใช้เทคนิคการวินิจฉัยภาพต่อมน้ำเหลืองบริเวณรักแร้ในผู้ป่วยมะเร็งเต้านม

กรณีศึกษาที่ 1
การวินิจฉัยการแพร่กระจายของโรคมะเร็งเต้านมไปยังต่อมน้ำเหลืองโดยใช้เครื่องอัลตราซาวด์ (Ultrasonography) ตรวจบริเวณรักแร้ วิธีนี้จะพบอัตราความผิดพลาดสูงถ้านำมาใช้กับผู้ป่วยมะเร็งเต้านมที่มีการแพร่กระจายไปยังต่อมน้ำเหลืองเพียงเล็กน้อย ถ้าหากตรวจพบการแพร่กระจายของเซลล์มะเร็งไปยังต่อมน้ำเหลืองโดยการวินิจฉัยภาพอัลตราซาวด์บริเวณรักแร้ ผู้ป่วยมักจะเป็นมะเร็งเต้านมแพร่กระจายไปยังต่อมน้ำเหลืองจำนวนมาก ในผู้ป่วยกลุ่มนี้วิธีการรักษาโดยให้เคมีบำบัดก่อนการผ่าตัดจะช่วยป้องกันการแพร่กระจายของโรคมะเร็งเต้านมและสามารถลดขนาดก้อนเนื้องอกให้เล็กลงก่อนการผ่าตัด

กรณีศึกษาที่ 2
ปัจจัยที่เกี่ยวข้องกับความเสี่ยงของการแพร่กระจายโรคมะเร็งได้แสดงไว้ในแผนภาพรูปแบบการคำนวณความเสี่ยงแบบกราฟฟิค (Nomograms) 3 รูปแบบ คะแนนการคำนวณรวมข้อมูลอายุของผู้ป่วย ขนาดของเนื้องอกในเต้านม จำนวนของเนื้องอก ชนิดของเนื้องอก และข้อมูลเกี่ยวกับการแพร่กระจายของเซลล์มะเร็งเข้ามาในหลอดน้ำเหลืองในเต้านมแสดงผลการประเมินออกมาเป็น 3 ลักษณะ ได้แก่การประเมินว่ามะเร็งไม่ได้แพร่กระจายไปต่อมน้ำเหลือง (0 ต่อม) มะเร็งแพร่กระจายเพียงเล็กน้อย (1-2 ต่อม) หรือมะเร็งแพร่กระจายระยะรุนแรง (3 ต่อม หรือมากกว่า) การศึกษาให้ทราบระดับการแพร่กระจายของโรคมะเร็งนี้มีผลสำคัญต่อการวางแผนการผ่าตัด

กรณีศึกษาที่ 3

ประเมินระดับการแพร่กระจายของมะเร็งสู่ต่อมน้ำเหลืองโดยการใช้เท็คนิคเครือข่ายประสาทเทียม (Artificial neural network ย่อว่า ANN) ศึกษาปัจจัยที่มีผลต่อการแพร่กระจายของมะเร็งเต้านมไปยังต่อมน้ำเหลือง เครือข่ายประสาทเทียมเป็นรูปแบบจำลองทางคอมพิวเตอร์ที่แสดงให้เห็นความสัมพันธ์ที่ซับซ้อนระหว่างองค์ประกอบของเนื้องอกกับความรุนแรงของโรคที่เพิ่มขึ้น รูปแบบที่สร้างขึ้นแสดงผลการประเมินออกมาเป็น 3 ลักษณะได้แก่การประเมินว่ามะเร็งไม่ได้แพร่กระจายไปต่อมน้ำเหลือง (0 ต่อม) มะเร็งแพร่กระจายสู่ต่อมน้ำเหลืองในจำนวนจำกัด (1-3 ต่อม) และมะเร็งแพร่กระจายระยะรุนแรง (4 ต่อม หรือมากกว่า) รูปแบบจำลองทางคอมพิวเตอร์นี้ให้ความถูกต้องมากกว่าการคำนวณทางสถิติแบบดั้งเดิมในการคาดคะเนความรุนแรงของการแพร่กระจายของมะเร็งไปยังต่อมน้ำเหลือง การประเมินความเสี่ยงที่มะเร็งจะแพร่กระจายไปยังต่อมน้ำเหลืองเป็นสิ่งสำคัญสำหรับผู้ป่วย ถ้าผลระบุว่าผู้ป่วยมีความเสี่ยงระดับต่ำที่มะเร็งจะแพร่กระจายไปยังต่อมน้ำเหลือง ผู้ป่วยเหล่านี้อาจไม่จำเป็นต้องตัดชิ้นเนื้อตรวจจากต่อมน้ำเหลืองเซนติเนล

กรณีศึกษาที่ 4
การศึกษาความสัมพันธ์ระหว่างการแสดงออกของยีนและการแพร่กระจายของโรคมะเร็งไปยังต่อมน้ำเหลือง ยีนที่มีความแตกต่างกันของมะเร็งเต้านมแต่ละชนิดจะมีความสัมพันธ์กับการแพร่กระจายของมะเร็งไปยังต่อมน้ำเหลือง ในกลุ่มมะเร็งชนิดที่ตอบสนองต่อฮอร์โมนเพศหญิงได้พบว่าการแสดงออกของยีนที่เชื่อมโยงกับการควบคุมการแบ่งตัวของเซลล์มะเร็งและการเจริญเติบโตของเนื้องอกมีอิทธิพลต่อการแพร่กระจายของเซลล์มะเร็งไปยังต่อมน้ำเหลือง ถึงแม้จะมีความรู้เกี่ยวกับอิทธิพลของยีนต่อการแพร่กระจายของมะเร็ง แต่การศึกษาได้พบว่าข้อมูลเกี่ยวกับขนาดของเนื้องอก และการทราบว่าเซลล์มะเร็งได้แพร่กระจายเข้ามาในหลอดน้ำเหลืองหรือไม่ ยังคงเป็นสิ่งสำคัญในการประเมินความเสี่ยงของการแพร่กระจายโรคมะเร็งไปยังต่อมน้ำเหลือง

ดุษฎีนิพนธ์ฉบับนี้ได้แสดงให้เห็นว่าปัจจัยที่เกี่ยวข้องกับผู้ป่วยและปัจจัยที่เกี่ยวข้องกับชนิดของมะเร็งเต้านมส่งผลกระทบต่อการแพร่กระจายของมะเร็งไปยังต่อมน้ำเหลืองและจำนวนต่อมน้ำเหลืองที่พบเซลล์มะเร็ง การใช้เครื่องมือและเท็คนิควิเคราะห์คาดการณ์ในปัจจัยเหล่านี้อาจช่วยประเมินถึงสถานะของต่อมน้ำเหลือง และช่วยปรับปรุงการรักษามะเร็งเต้านมโดยเฉพาะอย่างยิ่งการวิเคราะห์ระดับการแพร่กระจายของมะเร็งสู่ต่อมน้ำเหลืองจะสามารถลดขั้นตอนการผ่าตัดในรักแร้ที่ไม่จำเป็นและไม่ได้ให้ผลประโยชน์ใดๆในการรักษาผู้ป่วยกับช่วยลดผลข้างเคียงที่เกิดจากการผ่าตัด

Abbreviations

ALND	Axillary lymph node dissection
ANN	Artificial neural network
AUC	Area under the curve
AUS	Axillary ultrasonography
BMI	Body Mass Index
CI	Confidence interval
CNB	Core needle biopsy
ER	Estrogen receptor
FN	False negative
FNAB	Fine needle aspiration biopsy
FNR	False negative rate
FP	False positive
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
IHC	Immunohistochemistry
ITC	Isolated tumor cells
LNR	Lymph node ratio
LRR	Locoregional recurrence
LVI	Lymphovascular invasion
n	Number
N+	Any lymph node metastasis
N0	Lymph node negative
NHG	Nottingham histological grade
NPV	Negative predictive value
OR	Odds ratio
OS	Overall survival
PPV	Positive predictive value
PR	Progesterone receptor
RNAseq	RNA sequencing
ROC	Receiver operating characteristic
RT	Radiation therapy
SCAN-B	Sweden Cancerome Analysis Network–Breast
SLNB	Sentinel lymph node biopsy
TN	True negative
TP	True positive

Studies included in the thesis

The studies are referred to in the text by their Roman numerals.

I. The accuracy of preoperative axillary nodal staging in primary breast cancer by ultrasound is modified by nodal metastatic load and tumor biology.
***Dihge L**, Grabau DA, Rasmussen RW, Bendahl PO, Rydén L.*
Acta Oncologica. 2016;55(8):976-82.

II. Nomograms for preoperative prediction of axillary nodal status in breast cancer.
***Dihge L**, Bendahl PO, Rydén L.*
The British journal of surgery. 2017;104(11):1495-505

III. Estimating Disease-Free, Low-Burden and High-Burden Nodal Metastasis in Primary Breast Cancer using Artificial Neural Network Models.
***Dihge L**, Ohlsson M, Edén P, Bendahl PO, Rydén L.*
Submitted manuscript.

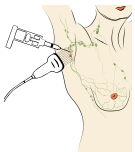
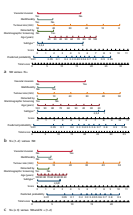
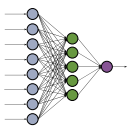
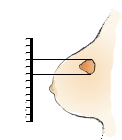
IV. Assessing Risk of Axillary Lymph Node Metastasis in Early Breast Cancer by Gene Expression and Clinicopathologial Models.
***Dihge L**, Vallon-Christersson J, Hegardt C, Saal L, Häkkinen J, Larsson C, Ehinger A, Loman N, Malmberg M, Bendahl PO, Borg Å, Staaf J, Rydén L.*
Manuscript.

Related studies not included in the thesis:

Completion axillary dissection can safely be omitted in screen detected breast cancer patients with micrometastases. A decade's experience from a single institution.
*Grabau DA, **Dihge L**, Fernö M, Ingvar C, Rydén L.*
European journal of surgical oncology: the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2013;39(6):601-607.

Epidermal growth factor receptor (EGFR) and the estrogen receptor modulator amplified in breast cancer (AIB1) for predicting clinical outcome after adjuvant tamoxifen in breast cancer.
***Dihge L**, Bendahl PO, Grabau DA, Isola J, Lövgren K, Rydén L, Fernö M*
Breast cancer research and treatment. 2008;109(2):255-262.

Thesis at a glance

Study	Research questions	Patients and Methods	Results and Implications
I 	Is the accuracy of axillary ultrasonography and ultrasonography-guided biopsy related to nodal metastatic burden and tumor biology?	473 consecutive patients diagnosed with primary breast cancer subjected for surgery without neoadjuvant treatment. Axillary ultrasonography and ultrasonography-guided biopsy was performed. Related cytology and clinicopathological data were retrieved.	The accuracy of axillary ultrasonography to detect nodal metastases is highly dependent on the size of the metastatic deposit and the number of involved nodes. Histological grade was found to modify the accuracy. Using axillary ultrasonography to detect low-burden metastatic disease warrants caution.
II 	Is it possible to use clinicopathological variables obtainable in the preoperative setting to develop decision-guidance tools to predict nodal metastatic burden?	692 consecutive breast cancer patients with T1-T2 tumors subjected for primary surgery. Logistic regression analysis was used to quantify the strength between the predictors and nodal metastatic burden. Internal validation was performed by bootstrap replications.	Nomograms including clinicopathological predictors of nodal disease demonstrated good abilities to discriminate: disease-free axilla, limited axillary nodal metastasis (1-2 positive lymph nodes), and heavy-burden axillary nodal metastasis (≥ 3 positive lymph nodes). If prospectively validated, the nomograms could facilitate preoperative decision-making regarding the extent of axillary surgery.
III 	Can artificial neural network (ANN) models based on preoperative obtainable clinicopathological characteristics distinguish patient groups with different levels of nodal metastatic involvement?	800 consecutive breast cancer patients with clinically node-negative axilla subjected for primary surgery. ANN-based models were developed based on 15 predictors of nodal status. Internal validation was performed by cross-validation.	ANN models proved superior to matching multivariable logistic regression models in predicting: disease-free axilla, N1 (1-3 nodal metastasis) and N2 (≥ 4 nodal metastasis). If prospectively validated, patients least likely to have nodal metastasis could be spared sentinel lymph node biopsy using the ANN model to predict disease-free axilla.
IV  	Can information on tumor gene expression alone or in combination with clinicopathological characteristics predict axillary nodal metastasis in different breast cancer subtypes?	3023 patients from the SCAN-B initiative subjected for primary surgery. Tumors were profiled by RNA sequencing. The performance of clinicopathological and gene expression-based predictors were assessed using machine-learning techniques. The predictors were evaluated in an independent validation cohort.	Addition of gene expression data to clinicopathological variables did not show a clear superiority in predicting nodal status. In cases with predicted nodal metastasis, proliferation-related genes were observed in ER+HER2- and HER2+ tumors, low expression of basal-like markers was detected for TNBC tumors. Futher studies investigating the performance of gene expression-based classifiers in more refined molecular subgrouping of breast cancer are warranted.

Historical perspective on surgical axillary lymph node management

The recognition

> *"When it possesses the breasts, it often causes inflammation to the armholes, and sends the swelling even to the glandules thereof"*

The above was written by the leading French barber-surgeon Ambroise Paré (1510–1590), who was among the first to recognize the spread of breast cancer to the axillary lymph nodes[1-3]. An early axillary surgical approach was recorded by the first president of the French Academy of Surgery, Jean Louis Petit, proposing excision of enlarged lymph nodes in the axilla[2,4].

Launching of en bloc clearance

In the 18th century, new practices developed with the introduction of axillary clearance[5]. An early suggestion on axillary lymph node dissection was proposed by Lorenz Heister, one of the founders of German surgery[6]. Nevertheless, the strongest argument to perform routine complete axillary lymphadenectomy came in 1867, when Charles Hewitt Moore recognized that lymph nodes affected by disease could remain clinically unrecognized[7,8]. Ernst Küster in Berlin systematically resected axillary fat together with the lymph nodes, even when the nodes were not palpable and appeared healthy[9]. The en bloc clearance of axillary lymph nodes together with breast cancer was further implemented by other surgeons, such as Richard von Volkmann, Joseph Lister, and Samuel D. Gross[6]. The work of these prominent surgeons had a great influence on William Stewart Halsted at the John Hopkins Hospital.

From Halsted to NSABP B-04

Halsted noticed that patients rarely developed cancer recurrence in the axilla if the axillary nodal dissection was routinely performed[2,6]. In 1882 he began the practice of "Halstedian" radical mastectomy, which profoundly revolutionized the surgical management of the breast and axilla[10]. Halsted's radical mastectomy included wide ablation with the removal of the mammary gland and overlying skin, the pectoral muscles, the entire transpectoral and axillary lymphatic tissue and the denudation of

the axillary vein (Figure 1). The wounds were left to heal by granulation, the patient's arm movements were vastly hampered, and lymphedema was ubiquitous. Although as many as three-fourths of his patients had axillary lymph node metastasis, the extensive axillary nodal dissection as part of this procedure yielded a 5-year survival rate of 40%[2,10]. The trend toward even more extensive surgery continued until the mid-twentieth century, which was exemplified by extended radical mastectomies and wider surgical resections aiming to encompass extra axillary nodes[11,12].

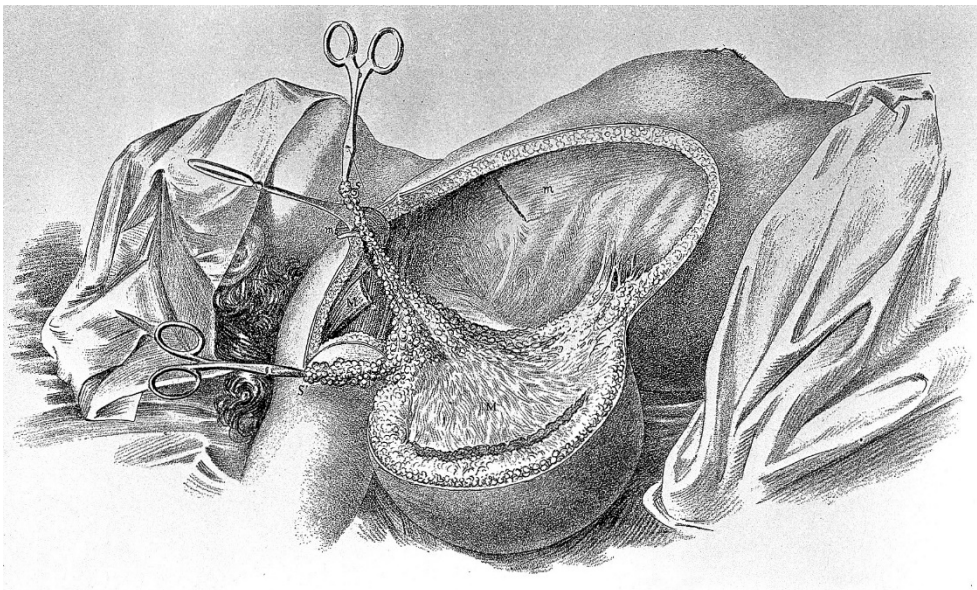


Figure 1. Illustration of Halsted's radical mastectomy, published in 1924.
'William Stewart Halsted, Surgical papers'. Credit: Wellcome Collection. CC BY

In 1948, Patey and Dyson published their work on the modified radical mastectomy[13] and suggested a more limited approach of surgical excision in breast cancer management. Not until the 1960s, following publications by Auchincloss[14] and Madden[15], who proposed to preserve both the pectoralis major and minor, and to separate the mammary gland from the skin flap by cutting Cooper's ligament, was a less extensive surgical management popularized. Auchincloss also argued for conservative axillary dissection.

Roughly a century after its introduction, the gold standard of axillary lymph node dissection for regional control and staging was challenged in one of the first clinical trials conducted by The National Surgical Adjuvant Breast and Bowel Project (NSABP B-04)[16], which sought to address the ultimate management of axillary lymph nodes. The study included breast cancer patients with clinically node-

negative disease and demonstrated that the initial omission of extensive axillary management by surgery or radiotherapy did not affect breast cancer survival. However, the risk of local recurrence was increased for those deprived of axillary treatment. Although the results suggested that not all metastatic lymph nodes were destined for clinical relevance, the routine surgical management of the axilla did not change and axillary lymph node dissection remained the standard of care.

The paradigm shift - Sentinel lymph node biopsy

In 1960, the concept of the "sentinel node" was launched by Ernest Gould and colleagues after the identification of metastatic involvement in an otherwise normal-looking lymph node[17]. The notion that the sentinel node could provide information on the status of the entire regional lymphatic basin has been attributed to the pioneering work of Ramon Cabanas published in 1977[18]. At this time, Donald Morton worked on mapping of sentinel nodes in malignant melanoma, and portrayed the intraoperative localization of the sentinel node using blue dye in 1992[19].

In breast cancer, the development of sentinel lymph node biopsy was described in the 1990s in a series of publications by Krag, Giuliano, and Veronesi[20-22], and is today the standard axillary staging procedure for clinically node-negative breast cancer patients.

Current challenges

With earlier detection, the overall node-positive rate in primary breast cancer has dropped dramatically from the 50-75% incidence of axillary metastasis in the first half of the twentieth century to 15-30% today. Even though nodal metastasis would be presented at diagnosis, only few nodes are involved. Thus, for the majority of newly diagnosed breast cancer patients, the invasive axillary staging has no therapeutic benefit. New techniques and refinements for axillary imaging have emerged alongside the advancement in the understanding of breast cancer biology. Contemporary randomized studies on axillary management have cast doubt on the benefit of extensive surgical excisions of axillary lymph nodes[23-27]. While major advances have been made since Charles Moore began routine axillary lymphadenectomy, axillary surgical staging is still performed in all clinically node-negative patients despite the notion that the majority has a disease-free axilla.

The selection of an adequate approach to axillary surgery or the choice to omit axillary staging would be facilitated by a preoperative diagnostic work-up that could enable accurate prediction of the axillary status. To improve axillary management in breast cancer, a better understanding of tumor biology related to metastasis is urgently needed. This knowledge, along with incorporation of axillary imaging technologies and risk assessing tools, could facilitate a more personalized axillary treatment.

Introduction

Breast cancer epidemiology

Globally, breast cancer ranks as the second most frequent cancer, but it is by far the most common cancer among women with approximately 1.7 million new cases every year[28]. In less developed regions of the world, breast cancer is the most common cause of cancer-related death, whereas in more developed countries, it is the second cause of death after lung cancer[28-30]. The documented incidence of breast cancer across the world varies widely; the lowest rates are found in Africa and Asia and the highest rates in the United States and Western European countries.

In the year 2016, 8923 invasive breast tumors were diagnosed in 7558 women in Sweden. This amounted to approximately 29% of all malignant tumors diagnosed in Swedish women during 2016[31]. According to The Swedish National Breast Cancer Registry, approximately 15% of the newly diagnosed breast cancer patients had node-positive disease[32].

During the last two decades, the breast cancer incidence in Sweden increased on average by 1.6% annually[31]. Approximately half of the cases were diagnosed in women before the age of 65[33]. Breast cancer was reported as the underlying cause of death in 1391 women in Sweden during 2016[31]. Differences in survival rates have been reported both within[34] and between different countries[28,35,36]. The international variations in breast cancer survival are partly due to differences in disease stage at diagnosis and differences in stage-specific survival[37]. In the majority of developed regions in the world, the age-standardized five-year net survival rates from breast cancer have increased gradually, reaching approximately 85% or more[38]. Breast cancer survival in Sweden is amongst the highest worldwide[35] with a 5-year survival rate of approximately 90%[31].

The breast

Development of the breast

Breast development is initiated in the five-week old fetus by the formation of the embryonal mammary ridge, milk line, and mammary buds. In a newborn, the breasts consist of undeveloped ducts that have small club-like termini which soon regress[39]. During infancy, the growth of the mammary glands is arrested. Until puberty, the breast is composed of lactiferous ducts without alveoli. Under the influence of the ovarian hormonal cascades during female puberty, the ducts start to proliferate. In this developmental phase, the ducts elongate, secondary branches of the ducts are formed, and their ends form the future breast lobules[40]. It is during the pregnancy and lactation phase that the mammary glands undergo full remodeling and growth. The cycle of induced mammary growth during pregnancy and lactation followed by involution is recurring with each pregnancy, and has been suggested to be protective against development of breast cancer[41]. Breast development is completed during post-menopause, when a phase of involution occurs. During this phase, glandular tissue atrophies, collagen degenerates, and connective tissue becomes less cellular[42].

Breast tissue and vascular supply

The breast is composed of adipose and glandular tissue, which is held together by a loose connective tissue framework called Cooper's ligaments[43,44]. The glandular tissue consists of lobes that are formed by lobules or clusters of alveoli. A ductal system drains the alveoli and larger ducts join to a main lactiferous duct that passes through the nipple (Figure 2).

The main blood supply to the breast is predominantly obtained from the anterior and posterior medial branches of the internal mammary artery and the lateral mammary branch of the lateral thoracic artery[45,46]. Even though the lateral thoracic artery supplies up to a third of the blood to the breast, there is wide variation in the proportion of blood supplied by each artery[47].

Lymphatic drainage

Lymph is taken up from the interstitial space into lymphatic capillaries with closed ends. These capillaries drain into collecting lymphatic vessels, which in turn drain into lymph nodes that are located along the lymphatic system[48]. Although the drainage pattern is highly variable[49], lymph from the breast is drained by two principal pathways: to the axillary lymph nodes and to the internal mammary nodes.

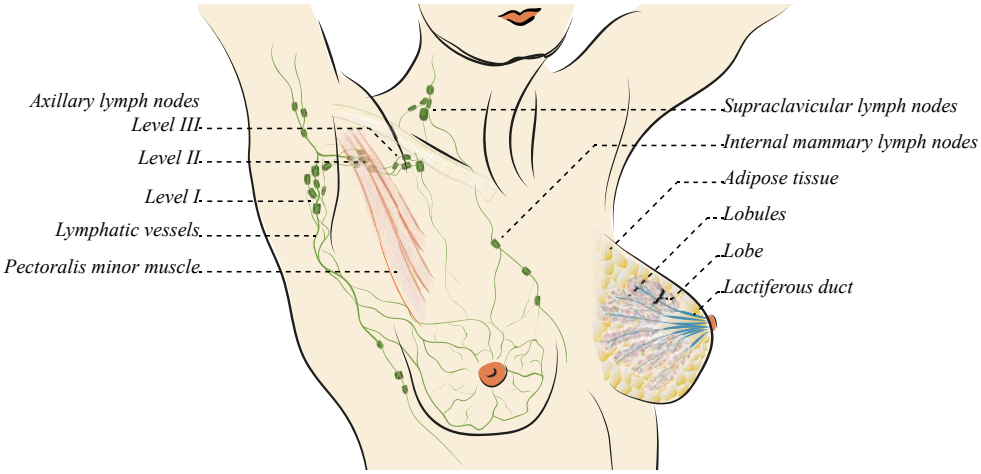


Figure 2.
Anatomy of the breast and overview of the axillary, internal mammary, supraclavicular lymph nodes, and the lymphatic vessels. The Level II axillary lymph nodes are located beneath the pectoralis minor muscle.

The main lymphatic drainage from the medial and lateral portions of the breast is to the axillary nodes[50], while the internal mammary nodes obtain lymph largely from the deep portion of the breast[51]. Blumgart et al. determined that lymphatic drainage from the breast to the axillary node field is most likely with a probability of 98.2%[51].

Drainage to the internal mammary, infraclavicular, supraclavicular, and interpectoral node fields occurs with a probability of 35.3%, 1.7%, 3.1%, and 0.7%, respectively, while drainage to multiple node fields occurs with a 36.4% probability[52]. Furthermore, drainage patterns between palpable and nonpalpable lesions differ[53]. Lymph drainage may change after surgical treatment in the breast or axilla, and an increased failure rate in axillary nodal mapping has been reported in breast cancer patients who have undergone previous treatment of the breast[54].

The axilla

Levels of axillary lymph nodes

The lymph nodes in the axilla are enclosed in a variable amount of adipose tissue. John W. Berg suggested that the axillary lymph nodes should be classified into three levels because the metastases from the breast cancer did not involve the axillary lymph nodes as an entity, but advance from level to level[55]. The categorization into three levels was based on their relationship to the pectoralis minor muscle (Figure 2). The Level I lymph nodes are the most numerous. These are located medial to the

latissimus dorsi muscle and inferior or lateral to the pectoralis minor muscle. Level II lymph nodes are those posterior to the pectoralis minor, below the axillary vein. Level III refers to the lymph nodes located medial to the pectoralis minor and against the chest wall. Metastatic involvement of these ipsilateral infraclavicular nodes confers a pN3 status[56].

Surgical anatomical borders

The anatomy of the axilla is intricate and consists of several nerves, arteries, and muscles. When performing axillary nodal dissection, the anatomical borders for the surgical field are the axillary vein superiorly, the serratus anterior and pectoralis minor muscles medially, and the latissimus dorsi muscle laterally. The axillary sheath encloses the brachial plexus, and the axillary artery and vein. With the dissection inferior to the axillary vein, the axillary artery and brachial plexus are kept superior to the surgical field[57-59]. The thoracodorsal artery and vein run with the thoracodorsal nerve and should be protected during axillary nodal dissection.

The long thoracic nerve innervates the serratus anterior muscle. Division of this nerve, which runs parallel to the chest wall, will result in a protruding "winged" scapula. The latissimus dorsi muscle is innervated by the thoracodorsal nerve. Injury to this nerve can cause weakness of arm abduction and internal rotation of the shoulder. The sensory intercostobrachial nerves course superficially in the axillary region and innervate the skin on the medial and posterior aspects of the upper arm, axilla, and posterior axillary line. If these nerves are injured, the patient could suffer from diminished sensation or hyperesthesia in the skin innervated by the severed branches of the nerve[60].

Diagnostic procedures in breast and axilla

Triple assessment

A suspicion of breast malignancy demands the gold standard by triple assessment for the initial work-up. This approach comprises radiological imaging of breast and axilla, physical examination, and cytology and/or biopsy. There is robust evidence for the value of the triple test due to its high overall sensitivity, which reaches almost 100% diagnostic accuracy[61-63]. The multidisciplinary care in the early breast cancer diagnostic work-up, as well as when the diagnosis of cancer is confirmed, enables treatment planning and more updated and efficient patient care[64].

Breast imaging

According to the report from the Swedish National Breast Cancer Registry, approximately 60% of breast cancer cases in Sweden were diagnosed by mammography screening in the year 2016[32]. Abnormal mammograms may be present in the majority, but not in all breast malignancies[65]. Mammographic sensitivity is particularly influenced by breast density. There are four radiological categories to describe breast density: 1) fatty; 2) scattered fibroglandular; 3) heterogeneously dense; and 4) dense. In the first two classifications of breast density, 85-90% of breast malignancies are identified by mammography. In the latter two categories, performance is lower, but has been increased with the transition from film to diagnostic digital mammography[66-68]. If mammography screening reveals abnormality, additional mammographic views and ultrasonography are used to portray a lesion more precisely. The diagnostic mammography, which is performed in patients with symptoms of breast cancer, is associated with a higher detection rate[69]. The radiological findings are summarized according to the American College of Radiology (ACR) BI-RADS (Breast Imaging Reporting and Data System)[70], suggestive of a normal, benign, or malignant diagnosis. Soft tissue masses and suspicious microcalcifications are the two main groups of mammographic findings indicative of a breast malignancy. If a spiculated lesion of the breast is revealed, it is in almost 90% of the cases representative of breast cancer[71].

Breast ultrasonography complements mammography in the diagnostic management. Ultrasonography is applied to differentiate soft tissue masses from cystic lesions and to provide guidance for biopsies. It is also the preferred methodology for imaging in younger women (< 35 years) with breast symptoms, and for women who are pregnant or breast-feeding[72,73]. Whole-breast ultrasonography has been reported to alter surgical management in up to 18% of women with mammographically detected malignancy due to its ability to identify additional multifocal or multicentric cancers and to complement the estimation of cancer extent[74-76].

Breast magnetic resonance imaging (MRI) has a high sensitivity in recognizing breast cancer lesions that are not manifested on clinical examination, mammography, or ultrasonography. However, the pooled moderate specificity could result in overtreatment[77]. Consequently, MRI is not recommended as a routine diagnostic work-up of breast cancer[78]. However, MRI screening should be applied for women with breast cancer gene mutations (e.g., BRCA 1 or BRCA 2) or those having similar risk[79,80].

Breast biopsy

The approaches for breast biopsy comprise skin punch biopsy, fine needle aspiration (FNA), core needle biopsy (CNB), and surgical biopsy. Additionally, assessment of nipple discharge or scraping cytology complements in the diagnostic evaluation. Percutaneous biopsy should be the approach of choice, followed by definitive surgery. Surgical biopsy is used only if percutaneous biopsy guided by palpation or imaging is not achievable, when repeated percutaneous biopsies are inconclusive, or if there are discordant results in relation to breast imaging[81]. In many centers, CNB has become the biopsy method of choice due to its ability to offer a more definitive histologic diagnosis, increased ability to analyze biomarkers, lower amount of inadequate sampling, and because it enables the possibility to distinguish between invasive and in-situ breast cancer[82,83].

Preoperative assessment of the axilla

For clinically node-negative patients or for patients without confirmed metastatic spread in a palpable node, sentinel lymph node biopsy and excision are standard for axillary staging. If preoperative noninvasive staging identifies abnormal axillary nodes and guided biopsy confirms the presence of axillary nodal metastasis, patients may proceed directly to axillary lymph node dissection rather than staging by sentinel lymph node biopsy.

Axillary clinical examination

The physical examination of the axilla is not sufficiently accurate to determine axillary lymph node status. While metastatic involved axillary lymph nodes are often nonpalpable, benign reactive lymphadenopathy may be clinically evident. The positive and negative predictive values of clinical palpation range between 61-84% and 50-60%, respectively[84-86].

Axillary imaging

Axillary characteristics suggestive of nodal metastasis may be seen on mammography. However, the ability to assess axillary lymph nodes in mammograms may be hampered by the limited visualization of the axilla. Ultrasonography is the modality of choice for axillary lymph node imaging[87]. Breast cancer patients with abnormal features of axillary lymph nodes by ultrasonography (e.g., changes in the nodal cortex or hilum[88]) have a higher risk of having multiple metastatic lymph nodes. The accuracy of axillary ultrasonography and guided biopsy is operator dependent, and there are wide ranging discrepancies between institutions[89].

There is an increasing interest in using MRI for preoperative axillary imaging. Some of the MRI benefits over ultrasonography include the ability of direct comparison with the contralateral axilla and less dependence on operator experience. Although a systemic review[90] has demonstrated that the performance of MRI to evaluate axillary status has been acceptable, this observation was based on studies with heterogeneous designs and small study cohorts. Moreover, there is to date no consensus on MRI criteria for the determination and reporting of axillary nodal appearance and status.

The utility of ^{18}F-fluorodeoxyglucose–positron emission tomography (^{18}F-FDG-PET) for axillary staging has revealed mixed outcomes. A multicenter prospective trial demonstrated a positive predictive value of 78-83% to identify axillary lymph node metastases when tumoral uptake of the marker involves multiple foci. However, the sensitivity to detect low-burden disease was only fair, 27%[91]. Moreover, given cost-effectiveness and radiation considerations, ^{18}F-FDG-PET/CT is likely to have restricted efficacy for axillary nodal staging[92,93].

Other novel methodologies for noninvasive axillary staging have been investigated. For example, magnetic resonance spectroscopy (MRS), which measures biochemical changes in tissue. ^{31}P MRS has been used for the noninvasive investigation of phospholipid metabolism, and suggested as a complementary technique to detect early nodal metastatic involvement and to reflect the nodal neoadjuvant therapy response[94].

Diffusion-weighted magnetic resonance imaging (DWI or DW-MRI) is a noncontrast MRI technique that assesses the capability of water molecules to freely diffuse in tissue. Several studies have analyzed the performance of DWI in evaluating nodal status[95-98]. However, published results have been inconsistent and further prospective studies are needed to confirm its utility.

Breast surgery

Mastectomy

A total or simple mastectomy consists of the removal of breast tissue and the nipple-areolar complex with an ellipse of skin surrounding it. Unlike the procedures of radical mastectomy and modified radical mastectomy, no attempt is made to involve the underlying pectoral muscles or the axillary lymph nodes[99]. Simple mastectomy and concomitant axillary staging are indicated for patients for whom the breast-conserving approach is not possible, for those who prefer mastectomy, and for

prophylactic intentions[100]. The options for breast reconstructive surgery should be offered all women undergoing mastectomy[101].

According to a multi-institutional study, factors that promote the choice of mastectomy were: preoperative MRI, impact of the individual surgeon, larger tumor size, higher nuclear grade, and patient age and ethnicity[102].

Breast conserving surgery

There are a limited number of absolute contraindications for the breast conserving approach, one of which is prior irradiation to the breast field[103]. For these patients, further postoperative radiotherapy would result in an excessive overall radiation dose to the thorax wall.

Breast conserving surgery and radiation therapy to eliminate subclinical disease in the breast (breast conserving therapy, BCT) is as effective as the complete removal of breast tissue, and results in similar long-term survival rates[104-107]. A meta-analysis of 17 randomized trials reported that radiotherapy versus no radiotherapy after breast conserving surgery reduced the 10-year risk of any recurrence with the absolute reduction of 16%[108]. The aim of breast conserving therapy is to provide a low rate of local recurrence and a satisfactory postoperative cosmetic result. In 2014, a meta-analysis concluded that 'no ink on tumor' should be a standard measure of negative margin, and that margins wider than recommended did not yield lower recurrence rates[109]. The increased risk of local recurrence following breast conserving surgery has been reported to be associated with young age, involved surgical margins, multicentricity, negative ER status, nodal-positivity, and absence of subsequent radiation therapy[110,111].

A positive nodal status is not a contraindication for breast conserving surgery, as breast conserving therapy and mastectomy have similar outcomes irrespective of metastatic nodal involvement. Moreover, a recent publication gave further evidence on the sufficiency of the current margin definition in patients with triple-negative tumors and node-positive disease[112].

Axillary surgery

Evaluation of the axillary nodal status guides treatment decisions in patients with breast cancer, and nodal status remains a key prognosticator[113].

Axillary lymph node dissection

Axillary lymph node dissection (ALND) remains the standard approach for patients with cytologically proven metastatic axillary lymph node(s). ALND upfront is also recommended for patients with inflammatory or locally advanced breast tumors with skin or chest wall involvement (T4)[78,114]. This recommendation is based on the hypothesis that the inflammation may cause obstructions in the lymphatics and yield unacceptable false negative rates at a SLNB[114,115].

Number of harvested lymph nodes

Boundaries for a typical ALND of level I and II lymph nodes are defined by anatomic borders as previously described (Chapter "The axilla"). More than 10 axillary lymph nodes are usually harvested during ALND. The amount of 10 or more harvested lymph nodes has previously been regarded as significant for accurate staging[116]. However, the impact of nodal status on the choice of adjuvant therapy is now highly debated (Chapter "Adjuvant systemic treatment"). Removal of level III axillary lymph nodes is not routinely performed. Incidence of skip metastases in the level III nodes with normal level I nodal status have been proposed to be approximately 3%[117]. A number of early publications have given evidence of the decreased risk of locoregional failure for patients with node-positive disease if ALND revealed a higher number of uninvolved nodes[118-121]. These findings may reflect a worse outcome for patients with understaging (few harvested lymph nodes), and who received undertreatment according to adjuvant treatment guidance at that time.

Sentinel lymph node biopsy

SLNB is the standard staging procedure for breast cancer patients with clinically node-negative axillary status[122]. Numerous investigations and meta-analyses have confirmed that the sentinel lymph node status accurately predict metastatic spread to the axillary basin[5,123-131].

The concept of axillary sentinel lymph node identification is based on the principle that malignant cells from a primary breast tumor metastasize to a limited number of lymph nodes ("sentinel" or "watchman" lymph nodes) before involving other nodes in the axilla[20,21]. The axillary lymphatic drainage from the entire breast reaches the same few sentinel lymph nodes[132]. Tracers (e.g., blue dye and radioactive colloid for standard dual mapping, superparamagnetic iron oxide, indocyanine green) injected into the breast enter the lymphatic vessels and drain into the sentinel lymph nodes (Figure 3). These nodes are subsequently identified by the presence of tracers and are generally found within the axillary level I[124].

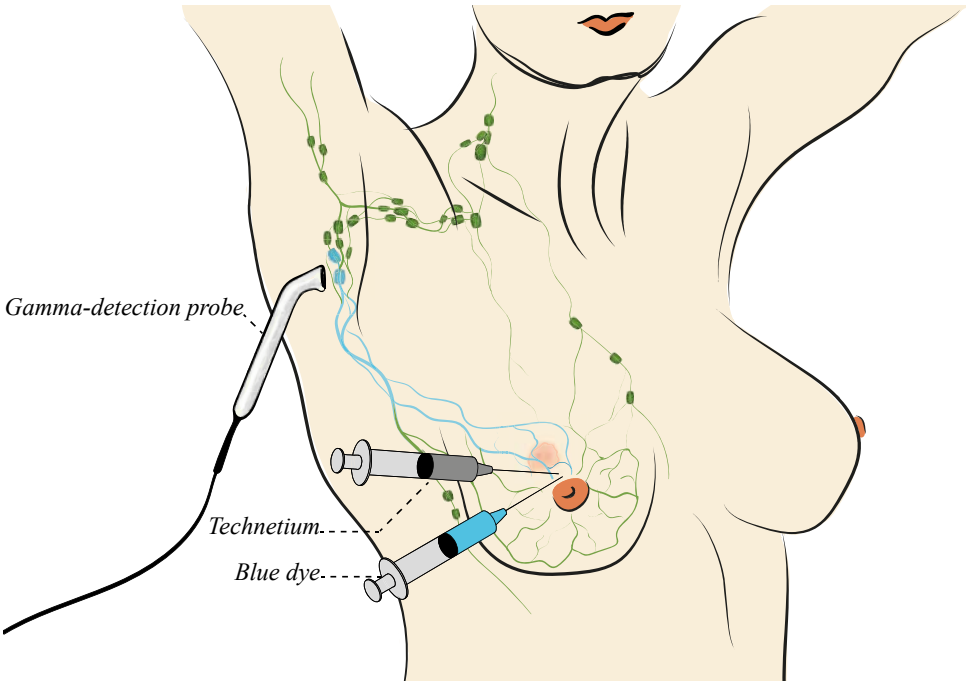


Figure 3. Sentinel lymph node mapping
Dual mapping with blue dye and radioactive colloid containing technetium identified by a gamma-detection probe.

False negative rate of sentinel lymph node biopsy

The false negative rate of SLNB is an important measure of procedural accuracy in the surgical management of breast cancer. The false negative rate (FNR) is calculated as the number of false negative cases divided by the number of all cases with axillary nodal metastasis (FNR = FN/FN+TP). FNR following sentinel node biopsy ranges between 5-10%[123,125,127,128,130,131,133,134] and decreases with surgical experience[135]. Although FNR of SLNB can be as high as 10%, the reported rates of axillary recurrence following a negative SLNB have been very low, ranging from 0.7-0.9%[133,136-138]. With more intensified systemic therapies over the last decade, the rates of axillary recurrence may even be lower than reported in the early studies.

Number of harvested sentinel lymph nodes

The optimal number of sentinel nodes that should be removed has been heavily debated. It has been proposed that the procedure should be terminated at the 4th node, since the only positive sentinel lymph node is hardly ever identified with increasing number of harvested lymph nodes (2% in >1000 patients)[139]. Other

publications have confirmed that removing more than four or five sentinel nodes did not increase the precision of the axillary staging[140,141]. The number of excised sentinel nodes has an impact on the false negative rate[142]. Previous data has revealed that 98% of node-positive patients were identified with the excision of the first three sentinel nodes[143]. A study, including 144,517 patients with T1-T3 tumors, showed that the adjusted disease-specific survival was better for patients with two or three excised sentinel lymph nodes than for those with only one sentinel node; the most favorable outcome was seen for those with three harvested sentinel lymph nodes[144].

Classification of lymph node metastasis and prognosis

Cancer cell deposits in axillary lymph nodes are categorized into clusters of isolated tumor cells, micrometastases, and macrometastases (Figure 4). The classification is based upon the size of the largest contiguous metastatic deposit in the lymph node[145].

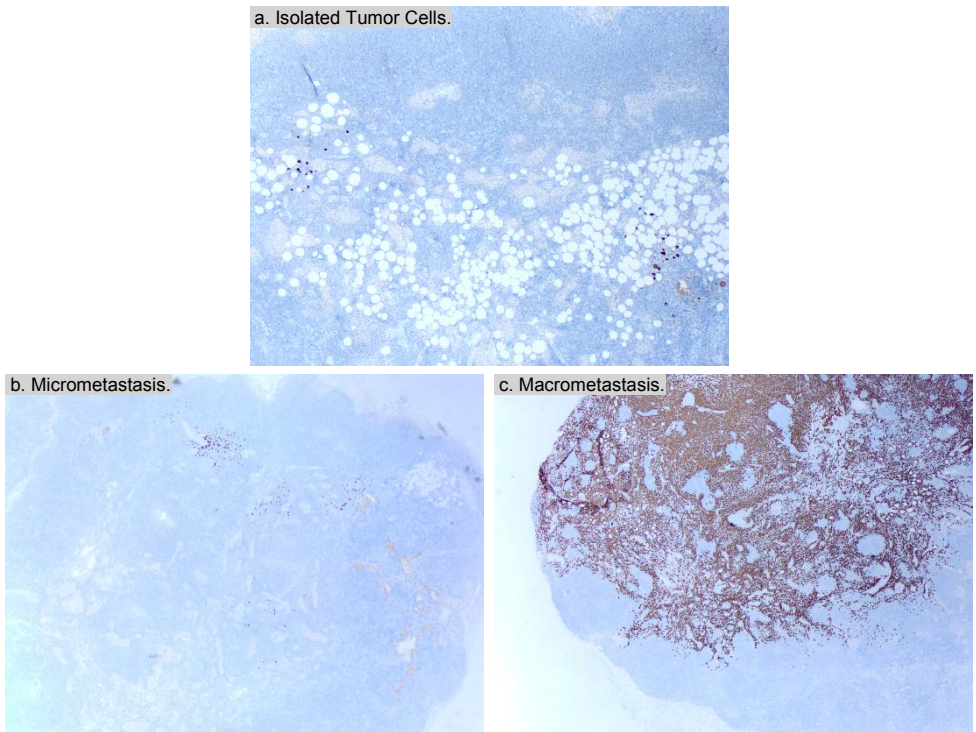


Figure 4. Metastatic deposits in axillary lymph nodes
a. Isolated Tumor Cells at 4x magnification; b. Micrometastasis at 2x magnification; c. Macrometastasis at 2x magnification. Images by courtesy of Anna Ehinger.

Isolated tumor cells - Isolated tumor cells (ITC) are clusters of tumor cells not larger than 0.2 mm or containing fewer than 200 cells in a single histologic lymph node cross section. ITC are designated as pN0(i+)

Micrometastasis - Micrometastatic lymph node involvement is defined as a metastatic deposit >0.2 mm but ≤ 2.0 mm or more than 200 cells.

Macrometastasis - Macrometastatic lymph node involvement of the axillary nodes is defined by a metastatic deposit >2.0 mm.

The significance of axillary lymph node status has long been recognized to affect the outcome of breast cancer[2,6-8], and has been verified as one of the most robust prognostic factors[146,147]. As such, positive nodal status has historically indicated adjuvant chemotherapy (Chapter ”Adjuvant systemic therapy”). Increased numbers of axillary lymph node metastases are strongly correlated to worse prognosis[148,149]. Clinical trials have categorized nodal involvement based on four nodal status groups in accordance with the National Surgical Adjuvant Breast and Bowel Project (NSABP) data: 0, 1-3, 4-9 and ≥ 10 metastatic lymph nodes. The 5-year survival rate for breast cancer patients with node-negative disease was presented as 82.8%, for 1–3 nodal metastases 73%, for 4–12 positive nodes 45.7%, and for ≥13 positive nodes 28.4%[149]. The categorization of nodal involvement 0, 1-3, 4-9 and ≥ 10 was further introduced in the guidelines of the St. Gallen Consensus Conference for primary therapy of early breast cancer in the year 2005[150]. Lymph node ratio (LNR) has been suggested as an alternative method to indicate prognosis, with a higher ratio of involved nodes designating a worse outcome. When high numbers of axillary lymph nodes are removed for pathologic analysis, LNR has been shown to estimate prognosis as well or better than the estimation made from the total number of positive lymph nodes[151-154]. In 2017, the American Joint Committee for Cancer (AJCC) expert panel concluded, however, that when only a few lymph nodes are excised for analysis, which often is the case with routine staging by sentinel lymph node biopsy, the LNR may be deceiving[56].

Since 2002, ITC and micrometastases have been distinct groups in the AJCC Cancer Staging Manual[155] because of uncertainty about the prognostic significance of ITC[156,157]. The cutoff value of 200 cells and 0.2 mm was, however, arbitrarily chosen[158]. Early studies presenting survival data on ITC and/or micrometastasis in sentinel lymph nodes has showed mixed results[159-163], which may have been due to different mode of detection. Prospective randomized studies have since addressed the prognostic relevance of the size of sentinel lymph node deposit in the context of axillary surgery and adjuvant treatment (Chapter “The required extent of axillary treatment”).

Adjuvant radiotherapy

Breast

The aim of adjuvant radiation therapy (RT) is to eliminate any residual tumor deposits after either breast conserving surgery or mastectomy to reduce the risk of locoregional recurrence and improve survival[108]. In 2011, a meta-analysis was published by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) on the benefits of whole breast radiotherapy succeeding breast conservation therapy. In comparison with breast conserving surgery alone, RT demonstrated a 50% reduction in the 10-year risk of any recurrence and a reduction in the risk of breast cancer death (absolute reduction 3.8%)[108].

Regional lymph nodes

For patients with negative axillary nodes on sentinel-node biopsy, the nodal recurrence rates are very low (<1%)[136]; thus, there is no rationale for regional nodal irradiation. Similarly, for those with micrometastatic nodal disease alone, there is evidence of excellent locoregional control by SLNB, whole breast RT, and systemic treatments[24].

RT to the regional lymph nodes is recommended for patients with macroscopically involved axillary lymph nodes. The NCIC-CTG MA.20 trial[164], which included 1832 women treated with breast conserving therapy for node-positive disease or high-risk node-negative disease, randomly assigned patients to receive nodal-RT or not in addition to whole breast RT. This trial concluded that the addition of regional nodal irradiation reduced the rate of breast cancer recurrence, but did not improve the overall survival at a median follow-up of 9.5 years[164]. Similarly, the EORTC 22922/10925 trial[165], with 4004 patients treated for early-stage breast cancer, revealed that RT of the regional lymph nodes had a minimal effect on overall survival. Nevertheless, there was an improved disease-free survival and distant disease-free survival, as well as a reduction in breast cancer mortality. While these trials revealed a 2.7% and 4.2% recurrence after ten years among those without regional radiotherapy, the American College of Surgeons Oncology Group (ACOSOG) Z0011 trial[23,27], included patients with 1-2 positive sentinel lymph nodes who received whole breast RT, and reported a regional recurrence rate of <0.5%.

The irradiation protocol for the Z0011 trial has been criticized[166], and the benefit of routine nodal irradiation for patients with low-burden axillary disease and low-risk features of the breast tumors remains debatable[167-169]. Toxicities associated with RT,

especially in older techniques, are cardiac toxicity, pneumonitis, risk of lymphedema, and cutaneous reactions[170].

According to the current Swedish National guidelines[171], omission of nodal irradiation can be considered in selected women with low burden nodal involvement and low-risk tumor features. Similarly, irradiation of the internal mammary lymph nodes may not routinely be included in the radiation field[171].

Adjuvant systemic therapy

Systemic adjuvant treatment refers primarily to the postoperative use of endocrine therapy, chemotherapy, human epidermal growth factor receptor 2 (HER2)-targeted treatment, and bisphosphonate therapy. The goal is to eliminate micrometastases and reduce the risk of recurrence.

Hormone receptor-positive breast cancer accounts for approximately 75-85% of all cancers[172,173]. For patients with early stage, ER+HER2- tumors, anti-estrogen therapy by selective estrogen receptor (ER) modifiers (SERM, e.g., tamoxifen) and/or aromatase inhibitors is the foundation of adjuvant treatment. A meta-analysis including data from 10,645 patients with ER-positive tumors showed that adjuvant tamoxifen treatment for five years significantly reduces the risk of breast cancer recurrence and breast cancer–related mortality by approximately 30%[174].

The first polychemotherapy to be tested in a prospective clinical trial was cyclophosphamide, methotrexate, and 5-fluorouracil (CMF).This pioneering trial also revealed the benefits of adjuvant chemo-regimens for reducing breast cancer recurrence in patients with positive axillary lymph nodes[175]. The EBCTCG meta-analysis from 2012 demonstrated the superior efficacy of taxane-based and anthracycline-based regimens. Polychemotherapy reduced the 10-year breast cancer mortality by about one third. Although the relative reduction in breast cancer mortality was not affected by nodal status, the absolute benefit was greater for patients with nodal metastasis due to their higher risks without chemotherapy[176]. Results from an EBCTCG analysis presented at The San Antonio Breast Cancer Symposium 2017 proposed that dose-dense chemotherapy could further decrease the risk of recurrence and improve survival[177]. Correspondingly, a meta-analysis recently observed that there were greater relative benefits from dose-dense regimens in studies with higher proportions of node-positive pre-menopausal patients[178].

Amplification of the HER2 is observed in 15% of all breast cancers in Sweden[32,179]. The first anti-HER2 drug approved for treatment of HER2+ tumors was trastuzumab. The use of this anti-HER2 monoclonal antibody together with

chemotherapy is associated with a significantly longer time to disease progression and improved overall survival[180-182].

Bisphosphonates reduce the activity of osteoclasts. A meta-analysis from EBCTCG concluded that there were significant benefits from the use of adjuvant bisphosphonates for early-stage, postmenopausal, breast cancer patients with reductions in recurrence and breast cancer mortality[183].

Impact of nodal status on adjuvant therapy

With increasing attention on the importance of tumor biology, there is adding evidence of clinical utility for various biomarker assays. However, the most important factors to guide the magnitude and choices on the specific treatment are still the status of ER, PR, HER2, and disease stage along with age[184]. Surgical axillary staging still influences recommendations on adjuvant therapy[185]. However, whether surgical staging is necessary for deciding on using adjuvant therapy is controversial[186,187]. Specifically, data have supported that omission of nodal staging would not modify the decision on adjuvant therapy in elderly patients with Luminal A-like, clinically node-negative T1 tumors[188].

According to the Swedish national guidelines for breast cancer[171], patients with axillary nodal metastasis should be considered for prolonged adjuvant endocrine therapy (10 years) in agreement with findings from large adjuvant trials[189,190]. Adjuvant chemotherapy is recommended for patients with Luminal B-like tumors and nodal metastasis, or Luminal A-like tumors and at least four positive lymph nodes. Postmenopausal patients with nodal metastasis are recommended bisphosphonate in addition to other planned adjuvant treatments.

Mechanism of nodal metastasis and disease progression

It has been hypothesized that the lymphatics are the primary route for breast cancer metastasis[191]. As the breast tumor increases in bulk, the intratumoral interstitial pressure rises. To achieve homeostasis, the interstitial fluid is therefore released into the surrounding tissue[192]. Excess fluid enters lymphatic vessels, which are considerably more permeable than the blood vessels[193]. The fluid then passes through the axillary lymph nodes where metastatic deposits can develop. The flow rate in the lymphatic vessels is 100-500 times slower than in the vascular vessels, and therefore, tumor cells can persist better in the lymphatics due to the reduced mechanical stress[194]. However, the amount of cancer cells entering the lymphatics as compared to the blood is unknown. There are, however, still controversies related to how metastasis to distant organs progresses; whether it is through an indirect

route via the lymphatic vessels that run into the venous system, or through a direct route through blood vessels that serve nodal metastases. In the last decades, the established notion of lymphatic vessels as passive conducts in the development of cancer metastases has been challenged[195]. Mechanisms of tumor metastasis through induction of tumor lymphangiogenesis was proposed in 2001[196], suggesting that lymphangiogenic growth is induced by factors produced in the cancer, such as VEGF-C and VEGF-D. Moreover, lymphangiogenesis around axillary lymph node metastases has been suggested to promote further metastasis[197,198]. Today, tumor-derived factors are considered to be of importance in rendering the lymph node receptive for disseminating cancer cells; some of these factors include antigens, growth factors, cytokines, and exosomes[199].

Viewpoints on regional control

While it is beyond doubt that systemic therapies improve breast cancer survival, even for patients with clinically localized disease[200], the significance of local therapy on the prognosis has been extensively debated. Punglia et al. discussed in a review article[201] three viewpoints of breast cancer progression. The first viewpoint is the "Halstedian" concept[10,202], which suggests that breast malignancy is initially a local disease and malignant cells are spread into bordering regions in a stepwise manner through the lymphatic system and then to the distant sites. Therefore, the Halstedian view commanded an aggressive surgical therapy, which included the removal of the regional lymph nodes for disease control. The second viewpoint was a "systemic" concept. Dr. Bernard Fisher introduced the theory of breast cancer as a systemic disease with the possible metastatic involvement of distant organs in an early phase of tumor development, often at the time of local breast tumor diagnosis[203,204]. According to the systemic view, tumors can be stratified into those that metastasize and those that will never spread to distant sites. Thus, the theory suggested only a limited or no effect of local control by axillary nodal dissection on breast cancer survival, but emphasized the significance of the early hematogenous spread to distant organs. The third viewpoint integrates features from the previous two viewpoints, proposing not only that nodal status is of prognostic importance, but also that the failure to achieve local control may permit metastatic spread at a later stage[205,206].

The required extent of axillary treatment

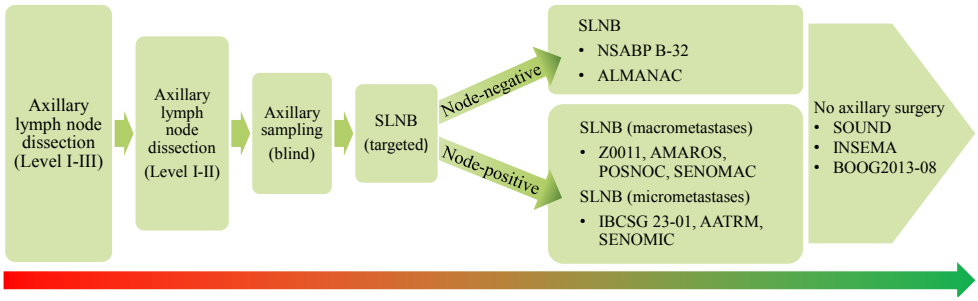


Figure 5. De-escalation of axillary lymph node surgery

Extensive surgery - no survival benefit

In the beginning of the 1970s, two large randomized trials challenged the hypothesis that the axillary lymph nodes were the only route for metastatic spread to distant organs.

In the cancer research campaign (King's/Cambridge) trial[207], 2800 breast cancer patients with early-stage, operable breast cancer were randomly assigned to mastectomy alone (with delayed axillary radiotherapy at signs of axillary recurrence), or mastectomy and axillary radiotherapy. The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-04 trial[208] assigned 1079 breast cancer patients with clinically node-negative disease to either mastectomy and axillary radiotherapy, mastectomy with ALND, or mastectomy alone with surveillance of the axilla (with delayed axillary nodal dissection for those with clinically overt axillary disease).

Although as many as 40% of patients at that time can be expected to have axillary metastatic involvement, the initial omission of axillary surgery or radiotherapy did not affect breast cancer survival at 10 years[207,208], or at 25 years of follow-up[209]. However, the risk of local recurrence was significantly increased for those allocated to conservative therapy of the axilla. These trials demonstrated the need to identify regional disease, but did not reveal any survival benefit from removing occult metastatic nodes[210].

Validity of sentinel lymph node biopsy

The validity of SLNB has been proven in several randomized controlled trials, which enrolled patients with early breast cancer and clinically node-negative axilla[22,127,133,211,212]. With the inclusion of 5611 patients with early breast cancer and clinically node-negative axillary status, the NSABP B-32 trial[133] was the largest of the randomized controlled trials to compare SLNB with ALND. Patients were randomized to SLNB alone, or SLNB with subsequent ALND, and those with positive SLNB underwent completion ALND. The end-points were disease-free survival, overall survival, regional recurrence, and postoperative morbidity. While the comparison in morbidity favored SLNB alone, there were no differences in the other end-points between the two groups. The European Institute of Oncology trial[123] compared SLNB only and SLNB with subsequent ALND, while the Axillary Lymphatic Mapping Against Nodal Axillary Clearance (ALMANAC)[127] trial assessed either SLNB alone, ALND, or nodal sampling. Altogether, these results reassured that SLNB is a proven methodology for patients with early, clinically node-negative, resectable breast cancer. For these patients, SLNB has succeeded ALND as the standard for axillary staging.

Limited metastatic involvement - limited axillary treatment

Isolated tumor cell

Isolated tumor cells in sentinel nodes can be detected using routine hematoxylin and eosin staining. While evaluation in serial levels and the use of immunohistochemistry (IHC) would increase the detection rate of occult metastases, results from previous publications[161,213,214] do not promote the routine use of IHC to look for occult metastatic deposits. A previous publication has suggested that the existence of ITCs in sentinel nodes yields a less promising prognosis than pN0 status. However, the prognostic difference was not seen for those with adjuvant systemic therapy[161]. Today, patients with ITCs only disease, pN0(i+) designation, are considered node-negative[215].

Positive sentinel nodes - micrometastasis

Two randomized controlled trials were conducted to evaluate if completion ALND was necessary when sentinel nodes only displayed metastatic deposits of 2 mm or smaller (micrometastasis). The International Breast Cancer Study Group (IBCSG) 23-01 trial[24] included women with T1-T2 primary tumors (70% T1 and approximately 90% < 3 cm in in the largest tumor dimension). Of the 1960 planned patients, 934 were included in this trial, which showed that omission of ALND was not inferior regarding its impact on prognosis. The 5-year disease-free survival was 87.8% without ALND and 84.4% with routine ALND. In the AATRM trial[216], 247

patients with tumors < 3.5 cm and sentinel node micrometastasis were assigned to either completion ALND, or no additional surgery. Similarly, there were no differences in disease-free survival. In the IBCSG 23-01 trial, about 90% of the patients had positive ER status. In both trials, only patients with clinically node-negative axillary status were eligible.

An ongoing Swedish multicenter study, SENOMIC, evaluates the omission of completion ALND, and includes patients with sentinel node micrometastases and larger primary tumors (T1-T3) than those accrued in the IBCSG 23-01 and AATRM trials. The outcome measures are disease-free survival and axillary recurrence rate.

Positive sentinel node - macrometastasis

Routine ALND after sentinel biopsy verified nodal metastasis has been challenged by the ACOSOG Z0011 and the AMAROS randomized controlled trials.

The American College of Surgeons Oncology Group (ACOSOG) Z0011 trial[23,27] addressed the need of completion ALND for patients with clinically node-negative T1-T2 breast cancer who had 1-2 sentinel nodes with macrometastasis. Eligible patients were treated with breast conserving surgery and radiotherapy to the whole breast, and received suitable adjuvant systemic therapy. In the SLNB with subsequent ALND study arm, merely one out of four patients had further metastatic involvement in the completion ALND specimen. Compared with the standard treatment, which includes completion ALND, SLNB alone resulted in an equivalent 5-year overall survival (91.9 % in the SLNB only group versus 92.5%), disease-free survival (82.2% in the SLNB only group versus 83.9%), and axillary recurrence. At a median 9.25 years of follow-up, the cumulative incidence of axillary recurrence rate was 1.5% in the SLND alone arm, in comparison with 0.5% in the completion ALND arm (p=0.28). The patients in ACOSOG Z0011 trial had mostly T1 (approximately 70%) and hormone receptor-positive tumors (85%). This trial has been criticized for an early closure due to slow accrual and low event rates, the fact that 20% of patients are lost to follow-up, and that high tangent radiotherapy was given to the axilla[166]. Even so, the results of the ACOSOG Z0011 trial has been practice-changing,[217-219] and the outcomes have been integrated into clinical guidelines[220]. A prospective validation study to the Z0011 showed that ALND could be omitted in a majority of Z0011-eligible patients with retained good regional control[221].

The European Organization for the Research and Treatment of Cancer (EORTC) conducted the After Mapping of the Axilla: Radiotherapy or Surgery (AMAROS) trial[25], which included 4806 breast cancer patients. Patients with T1-T2 tumors and clinically node-negative disease were eligible for inclusion. Of those with positive sentinel nodes, 744 were randomly assigned to pursue routine completion ALND, and 681 to obtain axillary radiotherapy. Almost all patients (95%) had 1-2 sentinel

node metastasis. The 5-year axillary recurrence rate was 0.43% in the ALND study arm, and 1.19% in the group who received axillary radiation group. No differences were observed between treatment arms regarding DFS or OS. However, the rate of lymphedema was higher for patients who underwent ALND than those with axillary radiotherapy at follow-up. As with the Z0011 trial, this trial has been criticized due to the low number of events and a short follow-up. However, the results presented by the AMAROS trial showed that axillary radiotherapy could be an option to ALND. The results from the AMAROS trial was further confirmed by the OTOASOR trial[222], showing that axillary radiotherapy in comparison with completion ALND was not inferior regarding recurrence and survival.

For patients who meet the Z0011-eligible criteria, regional radiation may not carry any advantage in comparison with whole-breast radiotherapy only. Two randomized controlled trials, NCIC MA.20[223] and EORTC 22922/10925[165], addressed the benefit of regional nodal radiotherapy in patients with axillary nodal metastasis who primarily underwent ALND. The results showed reductions in locoregional and distant recurrence, but no superiority in overall survival after radiotherapy.

Ongoing trials are further addressing the necessity of completion axillary treatment. SENOMAC is a prospective multicenter randomized trial with accrual of patients with T1-T3 breast cancer with clinical and ultrasonographic node-negative axilla and macrometastasis in 1-2 sentinel nodes. Patients are randomized between completion ALND and no further surgery. Since 2016, patients undergoing neoadjuvant treatment before SLNB are eligible for inclusion[224]. The Positive Sentinel Node: Observation vs Clearance (POSNOC) trial[225] includes patients with 1-2 positive sentinel macrometastases who are treated with either breast conservation surgery or mastectomy. Patients are randomly assigned to either adjuvant therapy alone or axillary treatment (ALND or axillary radiotherapy) and adjuvant therapy. These trials will also address whether the results from the ACOSOG Z0011 could be reproduced for patients treated with mastectomy.

Altogether, there is a further need to consolidate the findings for a more individualized nodal management with surgery, radiotherapy, and systemic adjuvant therapy.

Omission of axillary staging

Three ongoing randomized trials address the possibility to omit surgical axillary nodal staging; The Sentinel Node versus Observation after Axillary UltrasounD (SOUND) trial[26], the Intergroup-Sentinel-Mamma (INSEMA) trial[226] and the Dutch BOOG 2013-08 trial[227]. The noninferiority SOUND trial aims to compare SLNB with no axillary surgery. Patients eligible for the SOUND trial are women with T1 tumors with planned breast conserving surgery. If the preoperative axillary

ultrasonography or fine-needle aspiration cytology of a suspicious lymph node are normal, patients will be randomly assigned to either SLNB (with completion ALND if indicated) or no axillary surgical staging. The primary endpoint of the trial is distant disease-free survival. In INSEMA and BOOG 2013-08, patients with T1-T2 breast cancer and planned breast conserving surgery will be randomized to SLNB or no axillary surgery. In the INSEMA trial, patients with 1-3 nodal macrometastases in sentinel nodes will further be randomized to completion ALND or no further surgery, while those with four or more metastatic sentinel lymph nodes will undergo completion ALND. The planned sample size in the SOUND study protocol is 1560 patients, 5940 patients in INSEMA, and 1644 patients in BOOG 2013-08.

Clinicopathological characteristics and nodal metastasis

Patient-related characteristics

Age

The effect of age at diagnosis on lymph node metastasis has been studied in a large breast cancer patient cohort. This study recognized that patients aged ≤ 70 years were less likely to harbor nodal metastasis with increasing age, while those > 70 years of age were more likely to have a nodal-positive status with increasing age[228]. The underlying cause for this nonlinear association between age and nodal status, which has been confirmed by others[229], is not clear. One possible explanation could be that breast cancer is detected at a later stage in older patients after the end of regular mammography screening and patient omission of self-examination[230,231].

A more favorable tumor biology has been reported to be associated with advanced age[232,233]. Previous findings have shown limited survival benefits for surgical axillary management in older patients with small tumors and clinically node-negative status[188,234]. Nevertheless, others have shown that nodal metastasis in older patients occurred at an earlier stage in comparison to postmenopausal patients of a younger age[228]. Tumor-infiltrating lymphocytes in breast cancer decrease with age[235] and the risk of disease progression and recurrence has been suggested to be elevated in the elderly due to the decreased immunological response[236-238].

Body mass index and menopausal status

The relationship between Body Mass Index (BMI) and breast cancer risk is dependent on menopausal status. Among postmenopausal women, a higher BMI is associated with an increased breast cancer risk[239]. The relationship between an elevated BMI and postmenopausal risk of breast cancer may be related to the enhanced estrogen levels through conversion of estrogen precursors (from adipose tissue)[240]. Mechanisms related to hyperinsulinemia have also been suggested to enhance this risk[241]. For premenopausal women, however, higher BMI is associated with a reduced risk of breast cancer[242,243].

Previous findings on the relationship between BMI and nodal status in breast cancer have been contradictory. While some publications have suggested a higher risk of lymph node metastasis in association with increasing BMI[244-246], other investigators concluded that rates of sentinel node metastasis do not differ with varying BMI classifications[247]. Previous findings have proposed that obesity could increase the failure to localize the sentinel node. However, others have shown that these differences in the intraoperative sentinel node identification was of minor clinical relevance[142,248,249].

Mode of detection

The aim of mammography screening is to advance time of diagnosis so that earlier intervention can be undertaken to improve the outcome. Several randomized trials and meta-analyses have given evidence of mortality reduction related to mammography screening[250-255]. However, these findings of benefits of mammography screening are still debated[256]. Some authors maintain that mammography screening increases the incidence rates of nonlethal disease, but has a minor effect in reducing the incidence rates of advanced and potentially lethal cancers[256,257]. Others argue that one indicator of successful screening is the extent of node-negative disease at diagnosis.

A Swedish study investigated the effect of screening on disease stage at presentation and revealed a reduction of axillary metastatic involvement and stage II+ disease in comparison to the prescreening years[258]. Previous findings have also demonstrated that patients with breast cancer detected by screening have a higher likelihood of biologically low-risk tumors[259,260] and were more often sentinel node-negative than those with symptomatic presentation[261].

Tumor-related characteristics

Tumor size

Tumor size, defined as the greatest diameter of the breast tumor, has long been recognized to be associated with nodal status. The smaller a malign breast lesion is at diagnosis, the less likely it is to metastasize to the axillary lymph nodes[146]. Many studies have confirmed that the probability of nodal metastasis is highly related to tumor size[262-272]. Although tumor size and axillary nodal status are correlated, they are independent measures of outcome[146].

Lymphovascular invasion

Lymphovascular invasion (LVI), defined as the presence of tumor deposit within an endothelial-lined space in the breast tissue surrounding the invasive cancer[273] (Figure 6), has long been associated with lymph node involvement[262-264,266,269,272,274-276]. Since the detection of markers of specific lymphatic endothelial cells (i.e. D2-40 and LYVE-1), there is increasing evidence to support the correlation between lymphangiogenesis and breast cancer nodal and distant metastasis[277].

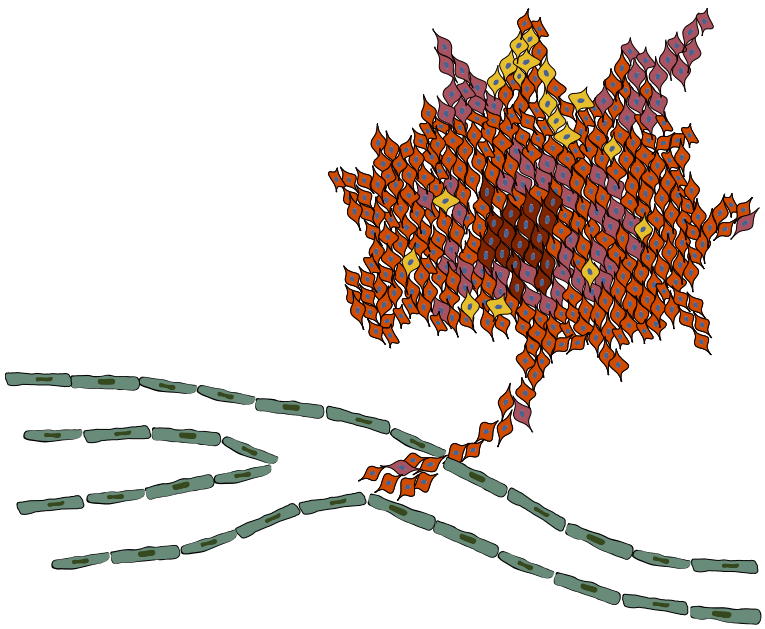


Figure 6. Lymphovascular invasion and lymphatic dissemination
Breast cancer cell invasion into a lymphatic vessel.

Multifocality/multicentricity

The presence of multifocality (foci of invasive tumors within the same breast quadrant) and/or multicentricity (foci of invasive tumors in separate breast quadrants) have been suggested as predictors of nodal metastasis[272,278-281]. Most of these findings are from studies accounting multifocal and multicentric cancers as an entity. Multicentric tumors have been suggested to be more aggressive than multifocal tumors and also more frequently associated with nodal metastasis[282].

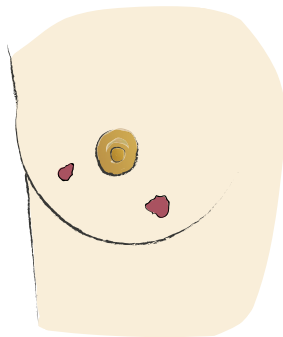


Figure 7. Multicentric breast cancer
Distinct foci of invasive tumors in different breast quadrants.

Hormone receptor status

The majority of breast tumors are ER-positive (approximately 85%)[179]. ER and progesterone receptor (PR) are members of the nuclear hormone receptor superfamily, which also includes the androgen and retinoid receptors, and are located in the cytosol. If activated by estrogen, ER translocates into the cell nucleus and operates as a ligand-dependent transcription factor. Analysis of ER and PR is performed routinely in all breast cancers by IHC. The cut-off levels have been varying from 1% to 10%[283]; the current St. Gallen guidelines define 1% positive nuclei as cut-off[113].

The reported associations between hormone receptor status and axillary nodal status have been inconsistent. While some studies suggest no predictive significance of neither ER nor PR status[266,276], others found that negative ER and PR status indicate a lower risk of axillary metastasis[265] or that PR status alone is inversely correlated with lymph node-positivity[263,272,284].

HER2 status

The HER-2 oncogene encodes for a transmembrane glycoprotein receptor of the epidermal growth factor receptor family with intracellular tyrosine kinase activity[285]. A routine part of the diagnostic work-up for breast tumors is testing for

HER2 using IHC to estimate overexpression, or in situ hybridization (ISH) to measure gene amplification[286]. HER2 is overexpressed in 15-30 % of all breast cancers worldwide[287] and it is correlated to worse prognosis[288] in the absence of HER2-directed therapies.

HER2 status has been proposed to predict lymphovascular invasion and axillary nodal metastasis[289,290]. While some publications have suggested that patients with HER2-positive tumors are more likely to display heavy metastatic disease burden (≥ 4 positive nodes)[291,292], others failed to show differences in lymph node positivity between HER2-negative and HER2-positive tumors[293].

Ki-67

Ki-67 is a protein which is expressed in mid-G1, S, G2, and M phases of proliferating cells, but not in resting cells of the G0 and early G1 phases[294,295]. Immunohistochemistry is often used to measure Ki-67 score, which is defined as the percentage of stained invasive cancer cells among the total number of malignant cells[296]. Heterogeneity in the assessment of Ki-67 values[297,298] have given rise to debates on the cut-off that should be applied to distinguish Luminal B-like from Luminal A-like[299]. The Ki-67 threshold of 14%[300], 20%[301], or within the range of 20%–29%[302] have been discussed by St. Gallen panels throughout recent years.

Two meta-analyses have shown the significance of Ki-67 as a prognostic factor[303,304]. Previous publications reported Ki-67 to be associated with nodal metastasis[305,306] and suggested Ki-67 as an independent predictor of level II axillary lymph node metastasis[305].

Molecular subtypes

Since the initial identification of breast cancer 'intrinsic' subtypes (basal-like, HER2-enriched, luminal, and normal breast-like) more than 17 years ago[307], subsequent studies have led to further sub-classification of luminal breast cancers[308]. Additional molecular subtypes are being proposed, including the claudin-low subtype, molecular apocrine subtype, and a novel luminal-like subtype[309-314].

Molecular classification based on immunohistochemistry and analyses of ER, PR, HER2, grade and Ki67 have provided predictive and prognostic values comparable with that of gene expression[315-317]. Correspondingly, clinicopathological surrogate definitions of breast cancer subtypes were proposed by the St. Gallen International Consensus in 2011[300] and endorsed in the guidelines for clinical decision-making in 2013[301]. The categorization into five molecular subtypes (Luminal A-like, Luminal B-like HER2-negative, Luminal B-like HER2-positive, HER2-type, Triple negative) helps to classify breast cancer patients into groups with different prognoses and treatment response[318].

The 2017 St. Gallen consensus panel reinforced the value of nodal status as a prognostic factor, regardless of the emerging amount of prognostic multigene assays. Although the panel acknowledged that gene expression profiles could improve the prognostication for node-positive breast cancers, the value of these signatures for making treatment decisions in node-positive disease is still controversial[113].

Associations between molecular subtypes and lymph node status in invasive breast cancer have been debatable. The triple-negative subtype, which is associated with worse prognosis, has been shown to infrequently metastasize to the axilla, while the triple-positive tumors have been suggested to be highly associated with nodal metastasis[289,319-325]

Histological grade

The histologic grading system most commonly used is the Nottingham grading system (Elston and Ellis modified Scarff-Bloom-Richardson grading system)[326,327] and is a valuation of differentiation. The system combines three morphological features (nuclear pleomorphism, tubular formation, mitotic count) and summed up to grades (I–III).

In an early publication, the incidence of nodal metastasis was shown to be 3% for patients with nonpalpable, small, low-grade tumors. Grade was suggested as an independent predictor of nodal metastasis[328]. Since then, subsequent studies have provided evidence for the association between high-grade tumors and axillary metastasis[263,275,329-332]. The association between grade and sentinel node status was, however, further evaluated by Viale et al. in a contemporary cohort comprising 4351 patients with relatively small primary tumors and clinically node-negative axilla. In the multivariable analysis, which included Ki-67, grade did not retain the association with sentinel node metastasis ascertained in the univariable analysis[272]. This confirmed previous findings on the collinearity between Ki-67 and grade in the association to nodal metastasis[333,334].

Histological type

Histological type signifies the growth pattern of the breast tumors. The most common is the invasive carcinoma of no special type, which accounts for 70-80% of all invasive breast lesions[335-337]. Although the terminology was changed in 2012, the terms ‘invasive ductal carcinoma’ or ‘invasive ductal carcinoma, not otherwise specified’ are accepted as alternative terminology options[336]. The most common specific subtypes include invasive lobular (10-15%). A classic lobular carcinoma is described as ER and PR positive, HER2 negative, grade II with low Ki-67[338]. Other histological types are tubular, cribriform, metaplastic, apocrine, mucinous, papillary, along with micropapillary carcinoma, and carcinoma with medullary and neuroendocrine types[339].

While previous publications have suggested higher proportions of nodal metastasis in lobular cancer than in the ductal type[340,341], other authors found no differences[342] or implied a lower incidence of metastasis[343]. Decreased probability of axillary lymph node metastases in patients with 'classic' invasive lobular cancer as opposed to the 'nonclassic' lobular subtypes has been described[344]. Furthermore, nodal metastasis from lobular carcinoma can be difficult to detect on hematoxylin and eosin staining, as it is comprised of non-cohesive cells of a comparable size to lymphocytes[345]. The risk of axillary lymph node metastasis in tubular cancers, which account for 2% of invasive breast cancers, has previously been shown to be very low[346,347].

Tumor localization

Breast cancer is likely to occur in the upper-outer quadrant localization of the breast[52,348-351] (Figure 8). Recently, a study showed that even though the upper-outer quadrant in general displayed the largest breast area and highest mean density, for patients with tumors in other breast quadrants, the density in that quadrant might not be the highest[351]. The authors concluded that there was no direct association between quadrant density and tumor localization. To date, the causes for the more frequently occurrence of breast tumors in the upper-outer quadrant is still not clear.

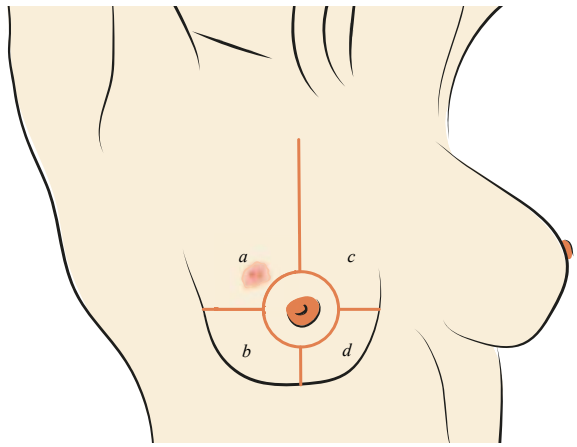


Figure 8. Quadrants of the right breast
Breast cancer is likely to occur in the upper outer quadrant localization of the breast.
a Upper outer quadrant; b Lower outer quadrant; c Upper inner quadrant; d Lower inner quadrant.

In a study cohort comprising 35319 patients from the Danish Breast Cancer Cooperation Group, Kroman et al. reported that the probability of a disease-free axilla was significantly greater for patients with medial T1-T2 tumors compared to those with lateral localized tumors. Although the same trend was observed for tumors > 5 cm, the difference did not reach statistical significance. This study

further showed, in line with previous findings from the Milan breast cancer center[352], that patients displaying tumors in the upper lateral quadrant had better prognosis compared to those with malignancy localized elsewhere in the breast. Recently, a contemporary study including approximately 100000 patients from the Chinese national cancer database confirmed the significance of tumor localization in the prediction of nodal metastasis[353].

Prediction tools in breast cancer care

Rationale

In clinical practice, risk stratification models assess the probability of the presence or absence of a given medical outcome based on the individual patient's clinical and nonclinical characteristics[354-356].

Breast cancer risk stratification models have been used for counselling and target prevention strategies for those with increased risk to develop the disease[357-361], to estimate neoadjuvant chemotherapy outcome[362] and to assess surgical outcome after surgery[363,364]. Numerous web-based multivariable models for prognostication and estimating the benefit as well as toxicity of intended adjuvant treatment have been proposed (e.g., Adjuvant online!, PREDICT, and CRASH)[365-369].

Nodal status dependent validity

Since the recognition of gene expression profiling in predicting clinical outcome for breast cancer patients[310,370], there is an increasing agreement that multigene tests could provide complimentary information on relapse-free survival to the standard routine prognostic factors such as tumor size, nodal status, and tumor grade. Today, most expert panels, including the American Society of Clinical Oncology, National Comprehensive Cancer Network, and the St. Gallen Consensus Group have endorsed the use of multigene prognostic assays in the decision-making on the use of adjuvant treatment in selected patients with primary breast cancer and ER+HER2- tumors[371,372].

The majority of the commercially available multigene assays have been discovered and validated in study cohorts with node-negative European and North American breast cancer patients 40-65 years of age[372]. Thus, the value of multigene assays for making treatment decisions in node-positive disease is still of controversy[113].

Recently, the randomized prospective MINDACT trial evaluated the utility of MammaPrint (70-gene signature) in individualizing adjuvant treatment regardless

of nodal status (0-3 metastatic lymph nodes). The trial suggested that the biologic features of the tumor are as significant as tumor burden with respect to choices on adjuvant therapies and patients' outcomes, even among those with limited nodal metastasis[373]. Today, increasing evidence supports prognostic value also in node-positive patients (1-3 metastatic lymph nodes) for the following multigene tests: Oncotype DX[374,375], MammaPrint[373], EndoPredict[376], and Prosigna[377]. The ongoing RxPONDER trial addresses whether adjuvant chemotherapy adds benefit to endocrine therapy for patients with ER-positive disease with 1-3 metastatic nodes, and a low-intermediate risk according to Oncotype DX recurrence score (RS $\leq$ 25).

Significance for axillary management

Prediction models are useful in the diagnostic work-up for assessment of the likelihood that a disease is present. Treatment is indicated when the likelihood of disease is high; if the risk of disease is low, the patient may be refrained from the intended therapy[378]. The same considerations are applied for preoperative decision-making; surgical interventions should only be given to those who benefit from the treatment. Still, although up to 85% of new-diagnosed breast cancer patients in Sweden present with node-negative disease and have excellent overall prognosis, all patients undergo surgical axillary staging[127,379,380]. Therefore, several risk assessment models/nomograms for estimating nodal involvement have been developed during the last decades, most of which aim to report the risk of additional non-sentinel node disease when SLNB proved positive[381-387].

The diverse accuracies of predictive models to estimate nodal disease involvement may reflect the complexity of factors linked to axillary metastasis[388-390]. However, other reasons for the moderate accuracies in validation studies could in part be associated with shortcomings in the model development, validation, and implementation[378,391].

Critical steps in prediction model development

Several reviews have revealed the quality of multivariable prediction models in medicine to be poor[392,393], and principle steps for prediction model development have been discussed in previous reports[355,378,394,395]. Recently, The Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) Initiative has suggested a set of recommendations for reporting of prediction models[396]. The main steps for model development can be summarized into: design of data collection, variable handling, selection of model technique, and evaluation of the predictive performance[378].

Aims

Overall Aim

To assess predictors of axillary nodal metastatic disease in patients with primary breast cancer, which can help develop prediction tools to estimate metastatic burden and facilitate preoperative decision-making regarding the extent of axillary treatment required for each patient.

Specific Aims

Study I To investigate the accuracy of axillary ultrasonography and ultrasonography-guided biopsy in relation to nodal metastatic burden, identify the modifying factors that affect technique precision, and compare axillary metastatic extent in patients diagnosed preoperatively with those diagnosed by sentinel lymph node biopsy.

Study II To develop nomograms by integrating clinicopathological variables obtainable in the preoperative setting for use in predicting the patient's nodal metastatic burden. This, in turn, can help facilitate the decision on extent of surgical axillary staging.

Study III To develop artificial neural network models based on preoperative obtainable clinicopathological characteristics that will help distinguish patient groups with different levels nodal metastatic involvement, and to identify candidates for sentinel lymph node biopsy omission.

Study IV To evaluate the potential of clinicopathological and gene expression-based predictors (alone and mixed) of lymph node metastasis to substitute or complement surgical nodal staging, and to assess the biological processes related to the predictors.

“Little p-value
What are you trying to say
Of significance?”

— Stephen Ziliak

Methods

Study population

Two main patient cohorts have been studied in this thesis.

Study I-III

The base-line cohort for Studies I-III consisted of all patients diagnosed with suspected breast malignancy between January 2009 and December 2012 at the Department of Surgery, Skåne University Hospital, Lund, Sweden, (n=1172). Diagnostic findings and decisions on surgical breast intervention and systemic treatments were discussed at the multidisciplinary breast tumor conference at Skåne University Hospital in Lund. The median age of the patients was 64 years, and the majority of the patients were presented with T1N0, Luminal A-like breast tumors detected through the public mammography screening program.

Exclusion criteria in all three studies were: other diagnosis than primary invasive breast carcinoma, omission of breast surgery as primary treatment, no surgical axillary lymph node staging, previous ipsilateral axillary surgery, and neoadjuvant treatment. Other specific exclusion criteria within each of the Studies I-III are outlined in the flowcharts (Figure 9).

Study IV

Study IV encompassed breast cancer patients diagnosed between September 2010 and March 2015 and enrolled in the Sweden Cancerome Analysis Network–Breast (SCAN-B) initiative. The patients were enrolled at any of the seven major hospitals in the Southern Sweden Healthcare Region (Lund, Malmö, Helsingborg, Kristianstad, Karlskrona,Växjö, and Halmstad) with a total catchment population of 1.8 million inhabitants. SCAN-B was initiated in 2010 with the goal to include all breast cancer patients in South Sweden for genomic analysis of the tumor tissue. Consecutive patient enrollment was part of routine breast cancer care, and the option for study inclusion was proposed to all newly diagnosed breast cancer patients in the catchment area[397,398].

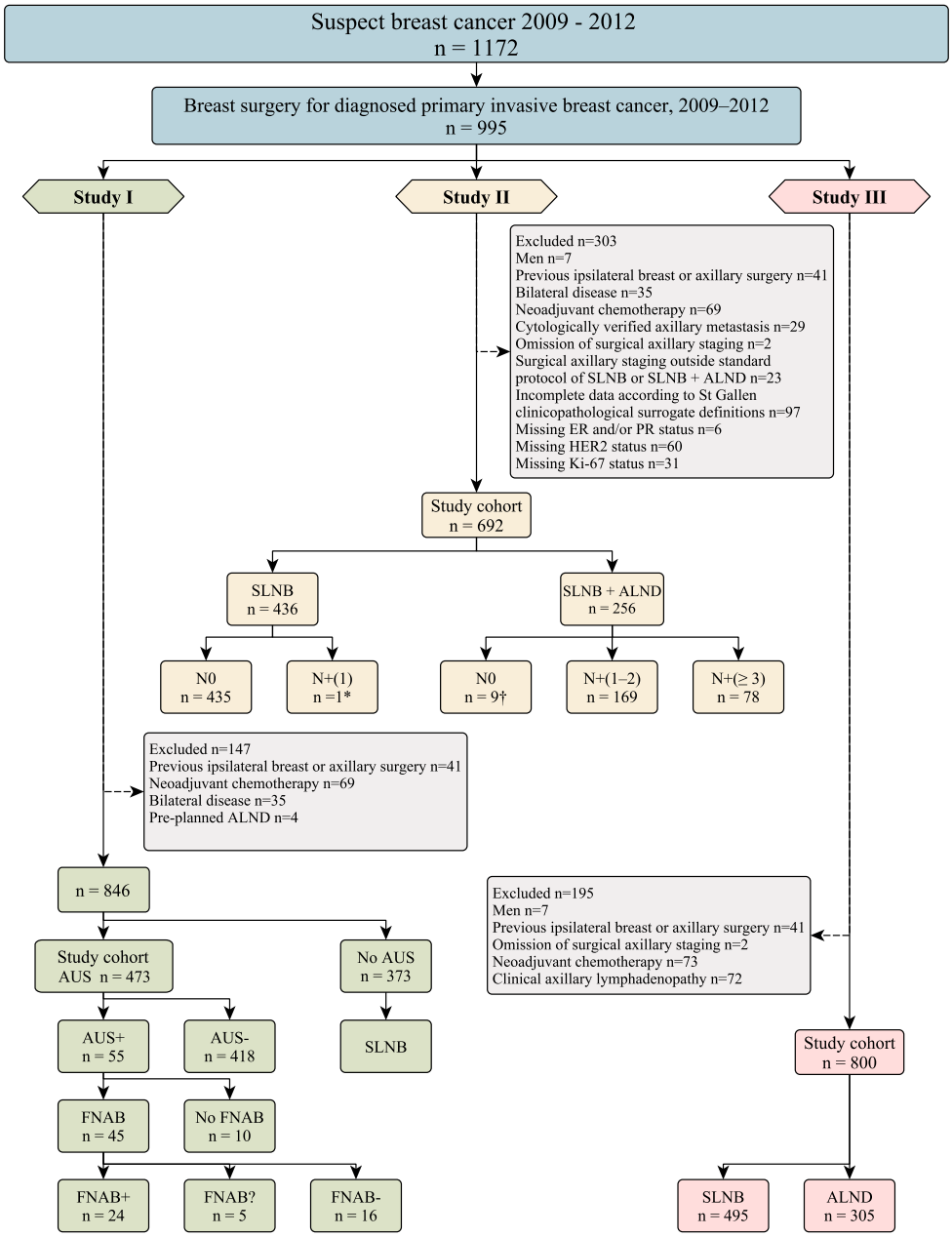


Figure 9. Flowchart and study overview, Studies I-III.
*Sentinel node biopsy (SLNB) showed solitary micrometastasis; false-negative frozen section of the involved node. †Presumed multifocality at the time of diagnosis; represents a selected group included in a study protocol with preplanned SLNB+axillary lymph node dissection (ALND). *AUS+*, Suspicious axillary ultrasound features; *FNAB+*, Malignant cytology; *FNAB?*, Uninterpretable cytology; *FNAB-*, Benign cytology; *N0*, lymph node-negative; *N+(1)*, solitary lymph node metastasis; *N+(1–2)*, lymph node metastasis involving one or two nodes; *N+(≥3)*, lymph node metastasis involving at least three nodes.

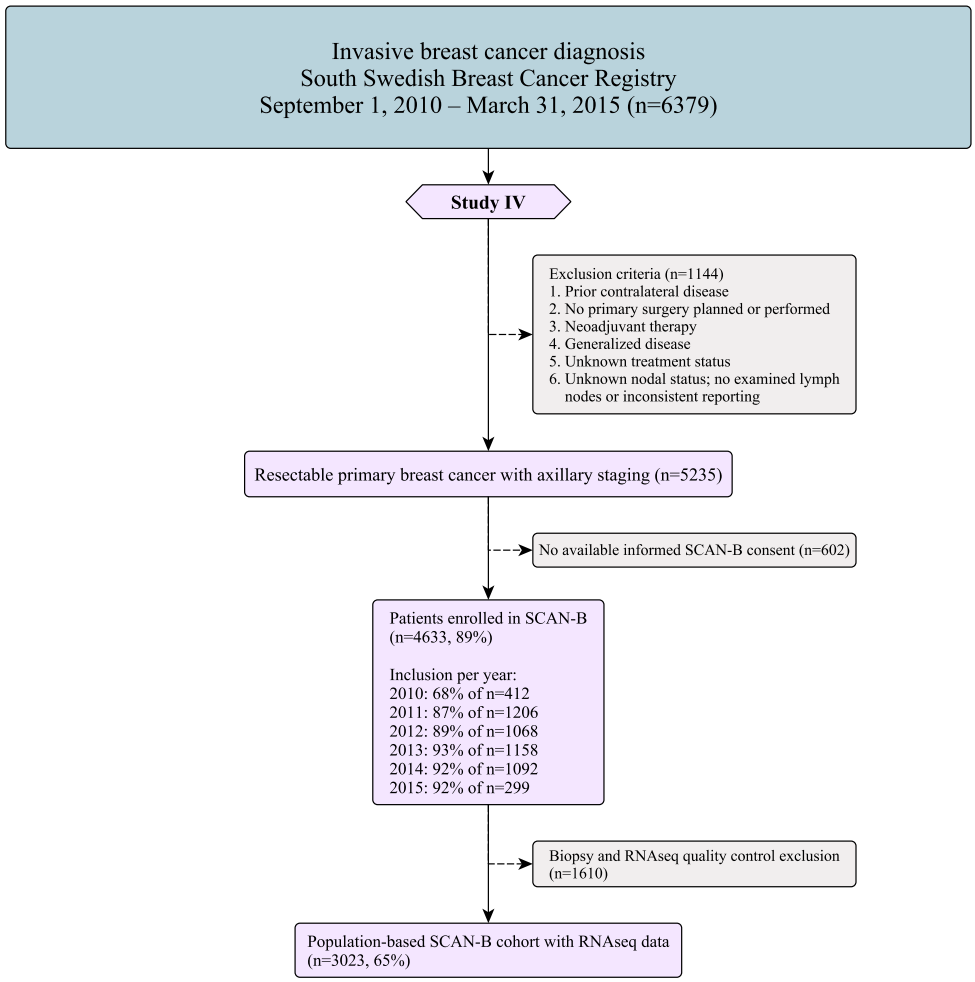


Figure 10. Flowchart and study overview, Study IV.
SCAN-B, Sweden Cancerome Analysis Network–Breast; *RNAseq*, RNA sequencing.

The study included 3023 patients with primary breast cancer and high quality RNA sequencing (RNAseq) of the breast tumor. The yearly rate of SCAN-B enrollment and exclusion criteria are displayed in Figure 10. Half of the tumors were detected through the public mammography screening program, and 65% of the patients were diagnosed with T1 tumors. The total cohort (n=3023) was divided into development (n=2278) and validation (n=745) cohorts. The validation cohort consisted of patients diagnosed in 2011 (median follow-up time of 5.8 years), while those diagnosed in 2010, 2012, 2013, 2014 and 2015 were assigned to the development cohort.

Study design and acquisition of clinicopathological data

Study I-III

Study I-III were designed as retrospective cohort studies, in which all patients were identified in a prospectively maintained pathology-based registry.

The Swedish National Quality Registry for Breast Cancer[33] was reviewed for information on previous breast and axilla surgery. Data retrieved from medical records included detailed preoperatively information on age at diagnosis, menopausal status, weight and height, tumor localization in the breast, mode of detection (dichotomized as screening or symptomatic presentation), and clinical axillary status. A breast pathologist extracted histopathological variables that included tumor size, multifocality, histopathological subtype, histological grade, status of ER, PR, HER2 and Ki-67, evidence of LVI, nodal status based on fine-needle aspiration biopsy (FNAB), and SLNB and/or ALND. The lymph node characteristics included the total number of examined nodes, number of metastatic nodes, and dimensions of the largest metastases (in mm and categorized into micrometastases and macrometastases). Radiographic appearance of the axillary lymph nodes by axillary ultrasonography examination was retrieved from a radiology database at Skåne University Hospital.

Study IV

Study IV is based on the multicenter SCAN-B initiative, which employs a prospective, population-based, observational cohort design (ClinicalTrials.gov ID: NCT02306096).

Information on age, tumor size, nodal status, mode of detection, multifocality, histological grade, LVI, status of ER, PR, and HER2, adjuvant therapies, and overall survival (OS) were retrieved from the Swedish National Quality Registry for Breast Cancer[33] and Statistics Sweden[399].

Axillary nodal status end-points

In Studies I-IV: N0 included node-negative cases and cases with ITC. The presence of micrometastases or macrometastases was defined as axillary node-positive (N+).

Table 1.
American Joint Committee on Cancer Definition of Pathological (pN) Regional Lymph Nodes[56].

Category	Criteria
pNX	Regional lymph nodes cannot be assessed (eg, not removed for pathological study or previously removed)
pN0	No regional lymph node metastasis identified or ITCs only
pN0(i+)	ITCs only (malignant cell clusters no larger than 0.2 mm) in regional lymph node(s)
pN0(mol+)	Positive molecular findings by reverse transcriptase-polymerase chain reaction (RT-PCR); no ITCs detected
pN1	Micrometastases; or metastases in 1-3 axillary lymph nodes; and/or clinically negative internal mammary lymph nodes with micrometastases or macrometastases by sentinel lymph node biopsy
pN1mi	Micrometastases (approximately 200 cells, larger than 0.2 mm, but none larger than 2.0 mm)
pN1a	Metastases in 1-3 axillary lymph nodes, at least one metastasis larger than 2.0 mm
pN1b	Metastases in ipsilateral internal mammary sentinel lymph nodes, excluding ITCs
pN1c	pN1a and pN1b combined
pN2	Metastases in 4-9 axillary lymph nodes; or positive ipsilateral internal mammary lymph nodes by imaging in the absence of axillary lymph node metastases
pN2a	Metastases in 4-9 axillary lymph nodes (at least one tumor deposit larger than 2.0 mm)
pN2b	Metastases in clinically detected internal mammary lymph nodes with or without microscopic confirmation; with pathologically negative axillary lymph nodes
pN3	Metastases in 10 or more axillary lymph nodes; *or* in infraclavicular (level III axillary) lymph nodes; *or* positive ipsilateral internal mammary lymph nodes by imaging in the presence of one or more positive level I and II axillary lymph nodes; *or* in more than 3 axillary lymph nodes and micrometastases or macrometastases by sentinel lymph node biopsy in clinically negative ipsilateral internal mammary lymph nodes; *or* in ipsilateral supraclavicular lymph nodes
pN3a	Metastases in 10 or more axillary lymph nodes (at least one tumor deposit larger than 2.0 mm); *or* metastases to the infraclavicular (level III axillary lymph) nodes
pN3b	pN1a or pN2a in the presence of cN2b (positive internal mammary lymph nodes by imaging); *or* pN2a in the presence of pN1b
pN3c	Metastases in ipsilateral supraclavicular lymph nodes

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The nodal status end-points for Studies II-IV (Predicting the risk of nodal metastasis and the metastatic burden) were:

Study II:

- N0 *versus* N+
- 1–2 positive lymph nodes *versus* N0
- ≥ 3 positive lymph nodes *versus* N0 and 1–2 positive lymph nodes

Study III:

- N0 *versus* N+
- 1-3 positive lymph nodes *versus* N0
- ≥ 4 positive *versus* N0 and 1–3 positive lymph nodes

Study IV:

- N0 *versus* N+

Body Mass Index

In Study I, BMI was dichotomized as BMI ≥30 kg/m^2 *versus* BMI <30 kg/m^2.

Table 2. WHO definition of Body Mass Index[400]
Definition for adults >20 years old.

Body Mass Index	
<18.5	Underweight
18.5 – 24.9	Normal weight
25.0 – 29.9	Pre-obesity
30.0 – 34.9	Obesity class I
35.0 – 39.9	Obesity class II
≥40	Obesity class III
$BMI = \frac{weight\ (kg)}{height\ (m) * height\ (m)}$	

WHO, World Health Organization

Surrogate definitions of breast cancer molecular subtypes

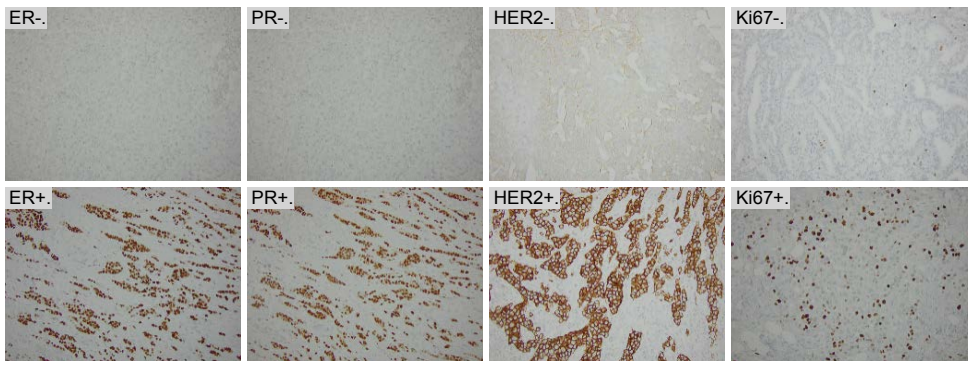


Figure 11.
Immunohistochemical staining for ER, PR, HER2 and Ki-67. Images by courtesy of Kristina Lövgren.

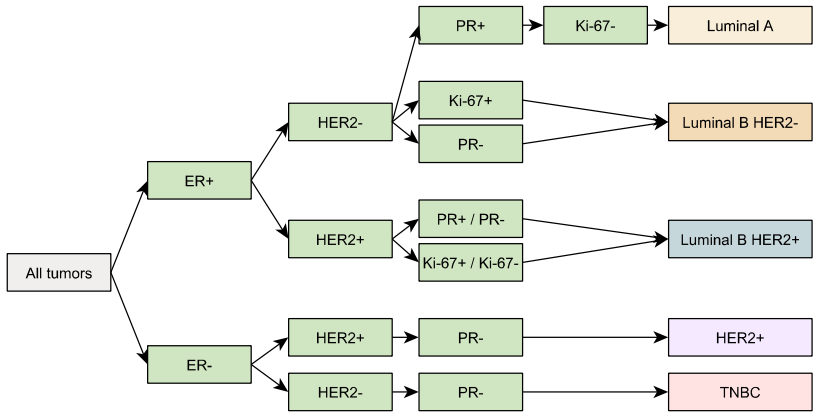


Figure 12. Surrogate molecular subtypes according to St. Gallen Guidelines 2013

In Study II, tumors were classified into five breast cancer subtypes based on the histopathological report of ER, PR, Ki-67 and HER2 status. The classification was according to the surrogate definitions adopted by the 2013 St. Gallen consensus[301]: Luminal A-like, Luminal B-like HER2-, Luminal B-like HER2+, HER2+ and triple-negative (Figure 12).

In Study IV, tumors were classified into three surrogate molecular subtypes based on the pathological report of ER, PR, and HER2 status: ER+HER2-, HER2+, TNBC.

Axillary ultrasonography

Ultrasonography techniques with higher frequencies have shorter wavelengths, are more easily absorbed, and therefore, do not penetrate as much as do techniques that use longer wavelengths [401]. Although imaging with high frequency wavelengths can provide enhanced spatial resolution, it comes at the expense of reduced depth of penetration[402], and thus, frequencies from 2 to 15 MHz are generally used in diagnostic radiology[403].

Technique

Axillary ultrasonography has increasingly become a part of the routine work-up in breast cancer diagnostics. Examination of the axilla is performed with a high frequency linear-array multi-frequency transducer. For patients with a higher BMI and larger axillary fat pad, a lower frequency setting may be required[87]. The linear transducer gives a rectangular field of view, a uniform beam throughout, and is utilized for imaging of superficial structures and small parts[404]. Color Doppler

ultrasonography may facilitate the detection of subtle abnormal blood flow within the cortex of the lymph node. All findings during the ultrasonographic assessment of the lymph nodes are suggested to be recorded in orthogonal planes[405].

The patient is often placed in a supine position with the ipsilateral arm abducted and the hand above the head[406]. Within the axillary level I, groups of lymph nodes can be visualized to follow the thoracodorsal and the lateral thoracic arteries. Isolated nodal groups may also be visualized within the fat pads in the axilla and axillary tail[407]. Although level II and III axillary levels are not routinely scanned, enlarged lymph nodes within level II may be observed. If clearly abnormal lymph nodes are observed in level I, certain institutional protocols endorse further scanning of the supraclavicular area and along the internal mammary artery[407].

Ultrasonographic imaging of the axillary lymph nodes in study I was performed using the Toshiba Aplio XG ultrasound system and a high-resolution multidimensional linear array transducer (7.2–14 MHz; PLT-1204AX).

Morphological findings related to nodal spread

A normal axillary lymph node has in general an oval or lobulated shape, a well-defined margin, and displays a hilar radiolucent notch[406]. The nodal cortex should be rather hypoechogenic and not thicker than 3 mm[408]. Typically, a normal lymph node is smaller than 2 cm in size in the long axis[408]. However, nodal size has a limited utility in differentiating metastatic involvement[409].

Breast cancer cells entering the lymph node through the afferent lymphatic may deposit in the subcapsular sinusoids[410]. The growth of these metastatic deposits can thus be visualized as thickening of the lymph node cortex or cortical bulge[411]. These morphologic changes of the nodes are regarded as the earliest detectable metastatic transformations that could be visualized on imaging, but could also be difficult to distinguish and are nonspecific[411]. Neovascularity adjacent to the subcapsular metastasis may result in aberrant cortical blood flow[412]. The spread of the cancer cells has been suggested to progress from the cortex to the paracortex and towards the deeper structures and medullary sinuses, and subsequently replaces the normal lymph node architecture[413]. Hilar obliteration could yield a completely hypoechogenic lymph node. While this may be the most specific sign for metastatic involvement, it marks an advanced disease[414]. Uncertainty persists as to which ultrasonographic features (e. g, cortical changes and vascularity) should be used to identify lymph node metastasis. In clinical practice, increase in cortical thickness of more than 3 mm has been shown to be the most useful predictor of metastatic spread[415]. Thus, classification systems accounting for changes in the lymph node cortex have emerged with the objective to estimate early nodal involvement[411,416]. There are to date no widely accepted standard definitions of ultrasonographically abnormal lymph node changes or widely accepted reporting guidelines. In study I,

a breast radiologist evaluated the overall morphological sonographic features of the axillary lymph nodes. Lymph nodes of any size were regarded as abnormal if changes in cortex or hilum were displayed (e.g., cortical thickening, cortical eccentricity, or nonexistent fatty hilum). All hypoechogenic nodes were classified as abnormal. Newer techniques, such as ultrasound elastography and contrast-enhanced ultrasound (CEUS) are further discussed in the Chapter "Future perspectives".

Ultrasonography-guided fine needle aspiration biopsy

Metastatic nodal features on ultrasonographic imaging are not pathognomonic for the presence of breast cancer nodal involvement, and there is an overlap with attributes of reactive benign lymph nodes. Accordingly, the sensitivity and specificity of axillary ultrasonography is moderate when applied alone. To increase diagnostic accuracy, lymph node ultrasonography is combined with puncture and/or biopsy[417,418] (Figure 13). The main objective of preoperative ultrasonography-guided percutaneous evaluation of the axillary lymph nodes has been to identify breast cancer patients with nodal metastasis, who receive neoadjuvant treatment, and those who could directly proceed to ALND, avoiding an extra surgical procedure with SLNB[415,419-423].

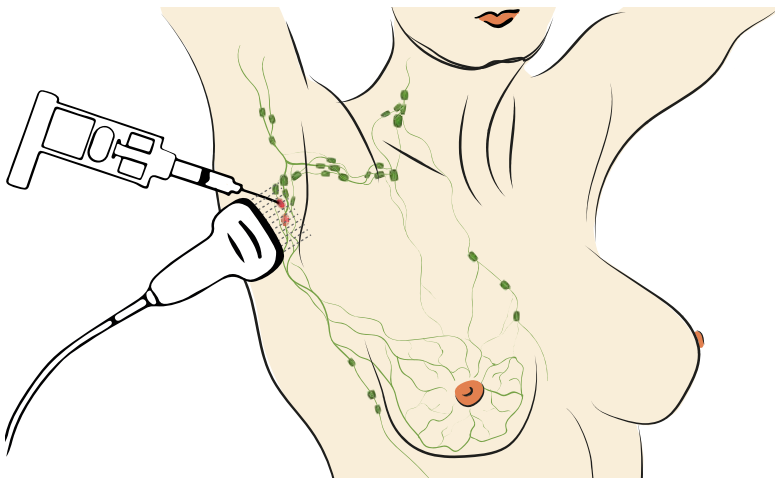


Figure 13. Ultrasonography-guided fine needle aspiration biopsy of axillary lymph nodes

Fine needle aspiration cytology (FNAC) or fine needle aspiration biopsy (FNAB) are two similar designations to describe a minimally-invasive tissue sampling by aspirating cells[424]. If properly performed, FNAB is a safe, rapid, and cost effective

procedure that can be performed in outpatient clinics[425,426]. To achieve adequate and representative aspirates, highly accurate execution of ultrasonographically guided FNAB is required, which depends on the person performing the procedure. Furthermore, a cytopathologist must be available. Many factors affect the success of the technique (e.g., the gauge of the needle used and the amount of aspirated samples). Moreover, there are intrinsic methodological limitations of FNAB, such as lack of histologic architecture[427]. There are few absolute contraindications to FNAB. With correct technique, complications in FNAB are quite rare with the most common ones being pain and hematomas[427].

In study I, sonographic guided FNAB was performed by direct aspiration with a 21-gauge, 50- mm-long needle (Braun Sterican®, Kruuse Svenska AB, Sweden) attached to a 10-ml syringe that was mounted on an aspiration device. The needle was moved in a fan-shaped motion to extract cells from the abnormal area of the targeted lymph node and was withdrawn without maintaining vacuum suction. The aspirated material was expressed onto glass slides on-site and alcohol-fixed smears were made for cytological analysis.

Cytological report of the fine needle aspiration biopsy

The reporting system used for breast FNAB in Study I is according to the European guidelines for quality assurance in breast cancer screening and diagnosis (C1-C5)[428]:

Table 3.
Diagnostic classification – Fine Needle Aspiration Biopsy

Classification	
C1	Unsatisfactory
C2	Benign
C3	Suspicious, probably benign
C4	Suspicious, probably malignant
C5	Malignant

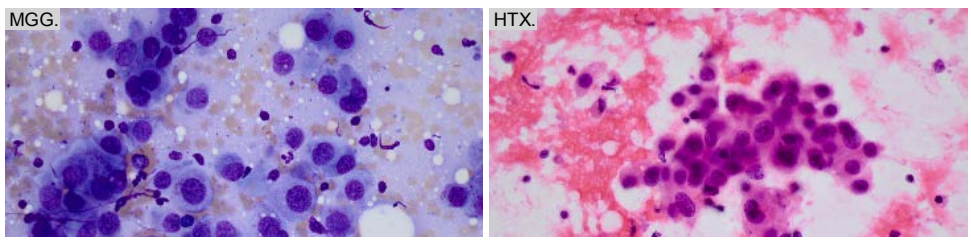
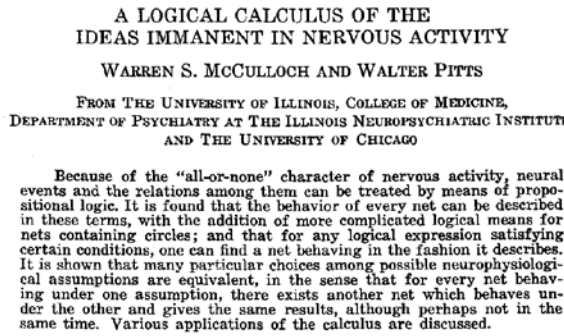


Figure 14.
C5 at 40x magnification, using May-Grünwald Giemsa (MGG), and Hematoxylin (HTX) staining, respectively. Images by courtesy of Anna Ehinger.

Artificial neural network



A LOGICAL CALCULUS OF THE
IDEAS IMMANENT IN NERVOUS ACTIVITY

WARREN S. MCCULLOCH AND WALTER PITTS

FROM THE UNIVERSITY OF ILLINOIS, COLLEGE OF MEDICINE,
DEPARTMENT OF PSYCHIATRY AT THE ILLINOIS NEUROPSYCHIATRIC INSTITUTE,
AND THE UNIVERSITY OF CHICAGO

Because of the "all-or-none" character of nervous activity, neural events and the relations among them can be treated by means of propositional logic. It is found that the behavior of every net can be described in these terms, with the addition of more complicated logical means for nets containing circles; and that for any logical expression satisfying certain conditions, one can find a net behaving in the fashion it describes. It is shown that many particular choices among possible neurophysiological assumptions are equivalent, in the sense that for every net behaving under one assumption, there exists another net which behaves under the other and gives the same results, although perhaps not in the same time. Various applications of the calculus are discussed.

Figure 15.
McCulloch, W.S. & Pitts, W. Bulletin of Mathematical Biophysics (1943) 5: 115.
Reprinted by permission from: Springer Nature, Bulletin of Mathematical Biology (A logical calculus of the ideas immanent in nervous activity, Warren S. McCulloch, Walter Pitts), Copyright 1943.

The publication by McCulloch and Pitt in 1943[429] is often taken to be the start for Neurocomputing and had great impact on further research on the development of brain-inspired processing units for computational purposes (Figure 15).

Artificial neural network (ANN) is a machine learning technique. It consists of several units of processing elements (artificial neurons or nodes). Although each unit can achieve simple information processing, the computational power of ANN comes from the linking of the processing nodes into a network, corresponding to the complexity of the biological synaptic connection[430]. ANN is effective in multifactorial analysis and holds the ability to explore underlying nonlinear interactions of interconnected predictors; it complements a traditional statistical approach[431]. Thus, ANN has been proposed as a supplement to standard statistical models for predicting multifaceted biological events[432-434] and has attained utilities in various clinical settings[435], in estimating diagnosis and prognosis of cancer[436-438], and for prediction of surgical outcomes.

Processing unit

A processing unit obtains input signals which are adjusted by connection weights (Figure 16). The weighted sum of the input signals passes through an activation function and produces an output. Each processing unit can obtain excitatory or inhibitory inputs, which further regulate the summing of the signals. In this way, a processing node is essentially an equation that balances inputs and outputs, and the activation function converts the input signals to output signals[439]. Several activation functions exist (e.g., step or threshold, sigmoid, piecewise linear, and Gaussian). The continuous, non-linear, sigmoid activation function is applied in Study III.

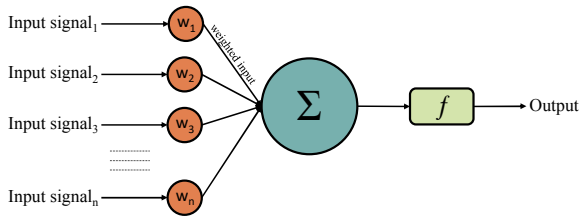


Figure 16. Processing unit within an artificial neural network

The main elements in the ANN architecture

Typically, the architecture of the ANN can be divided into three main elements or layers as displayed in Figure 17: (1) *the input layer*, which obtains information from the data set (2) *the hidden layer(s)*, which perform the internal analysis of a specific problem (3) *the output layer* which produces the concluding output from the achieved problem solution process[439]. The amount of hidden layers affects the number of neuron connections. During the problem solving process and network training, each input signal is tuned by the connection weight. Thus, too few connecting artificial neurons (nodes) in the hidden layer may hamper prediction performance, while an exceeding number of hidden nodes may result in overtraining/overfitting, with too complex a network tending to over-fit the solution.

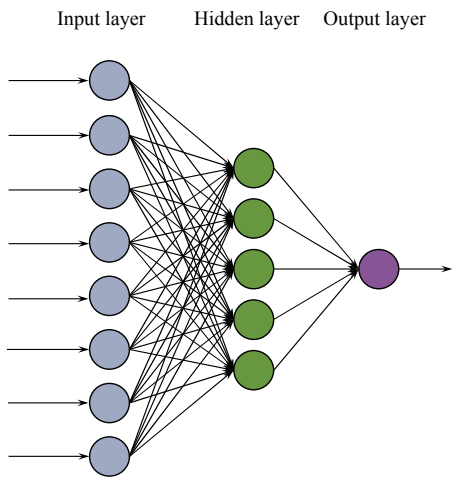


Figure 17. Feedforward artificial neural network with one hidden layer

Feedforward multi-layer perceptron and back-propagation

One of the most employed architectures for ANN is the multi-layer perceptron (MLP) feedforward neural network. Feedforward indicates that the influence of the input features, after being applied to the input layer, propagates through the hidden

layer(s) of the neural network until it reaches an output. Backwards propagation of error is one of the most popular techniques for error-correction learning. The network output is evaluated against the target output, and the error signals are iteratively transmitted backward. Most "learning rules" of the ANN are achieved by tuning the weights of the neuron connections and minimizing the error function[440]. While mean squared error (MSE) is often applied for regression problems, cross-entropy (CE) is an error measure often applied to quantify the error between the training network outputs and the preferred target outputs for classification problems[432,441].

ANN architecture in Study III

An ensemble technique was applied in study III. Twenty ANN ensemble models were trained and evaluated for each of the nodal status outputs (N0, N1 and N2); each ensemble consisted of 15 individually trained MLPs, where the average of these networks was used as the ensemble output. To avoid overfitting, the dropout technique[442,443] was employed on the input layer. Each of the MLPs was allowed to vary in size during model selection together with the amount of dropout used.

Dropout randomly sets the activities of processing units to zero, the processing unit and its connections are dropped from the artificial neural network during training. This procedure precludes the nodes from co-adapting and the technique is thus effective in preventing overfitting[442,443] (Figure 18).

For the final networks used during model validation, the hidden layer consisted of three nodes (median value), and dropout probabilities (in the input layer) were on average 0.6 for N0 and N1 models, while on average smaller for N2 (0.35).

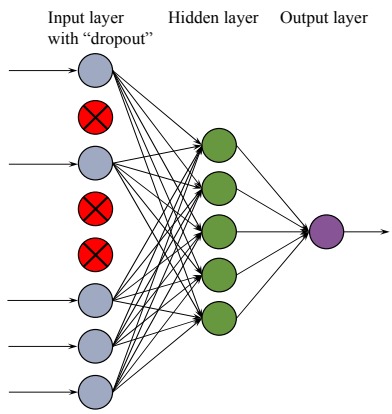


Figure 18. Dropout technique employed in the input layer
Dropout randomly sets the activities of processing units to zero; the unit and its connection are dropped from the artificial neural network.

Gene expression

Transcriptome studies enable insights into the fundamental elements of cancer development[444,445]. The two main contemporary techniques for transcriptome profiling are microarray-based methods and RNAseq. Microarray has been the technology of choice since the mid-1990s for large-scale gene expression studies, and the general principle is to quantify transcripts (genes) by hybridization to an array of predefined nucleotide oligomers (probes)[446,447]. The earliest work on RNAseq was reported in the mid-2000s, demonstrating the possibility to capture and quantify transcripts by direct sequencing due to the capabilities of high-throughput approaches. RNAseq can provide an outline of the complete transcriptome or messenger RNA (mRNA) profile at the given moment[444,448].

Acquisition of quality controlled RNAseq data

The association of gene expression profiling with lymph node status was studied in Study IV. Tumor RNAseq data was generated according to the protocol within the SCAN-B initiative as previously described[397,398]. In brief, fresh breast tumor specimen, taken by a breast pathologist, were stored cooled in microtubes with the preservative solution (RNAlater) before delivery to the central SCAN-B laboratory in Lund for tissue sample treatment and RNAseq. The sequencing library protocol is an adaptation of the dUTP method. In general, the depth of coverage refers to the number of times that a certain genomic site is sequenced during a sequencing run; data is of higher quality with the higher number of times that a base is sequenced[449,450]. Sequencing was performed to a depth of approximately 30 million paired-end reads (Illumina HiSeq2000 or NextSeq500 systems). The quality of RNAseq data was assessed; aligned reads < 10 million, duplication level > 55%, or RNA Integrity Number (RIN) <6 were excluded.

To correctly estimate gene expression, read counts must be normalized to adjust for systematic variances. Fragments per kilobase of exon per million reads mapped (FPKM) is a method for normalizing variances in read counts over genes or transcripts for paired end-reads[451]. A gene was represented by a single combined FPKM value of the matching transcript. An offset of +1 was added to all FPKM values followed by log2 transformation. A laboratory information management system (LIMS) and a data analysis and tracking system (BASE)[452,453] were used for the storage and processing of information.

Nodal status predictors in different surrogate molecular subtypes

In Study IV, tumors were classified into surrogate molecular subtypes based on the ER, PR, and HER2 status. Seven machine-learning models were applied to evaluate the predictive performance of nodal metastasis (N0/N+) for the entire development cohort and for the surrogate molecular subtypes. The models applied were: generalized linear model, boosted generalized linear model, random forest, generalized boosted regression models, partition around medoids, k-nearest neighbors algorithm and support vector machine) (Figure 19).

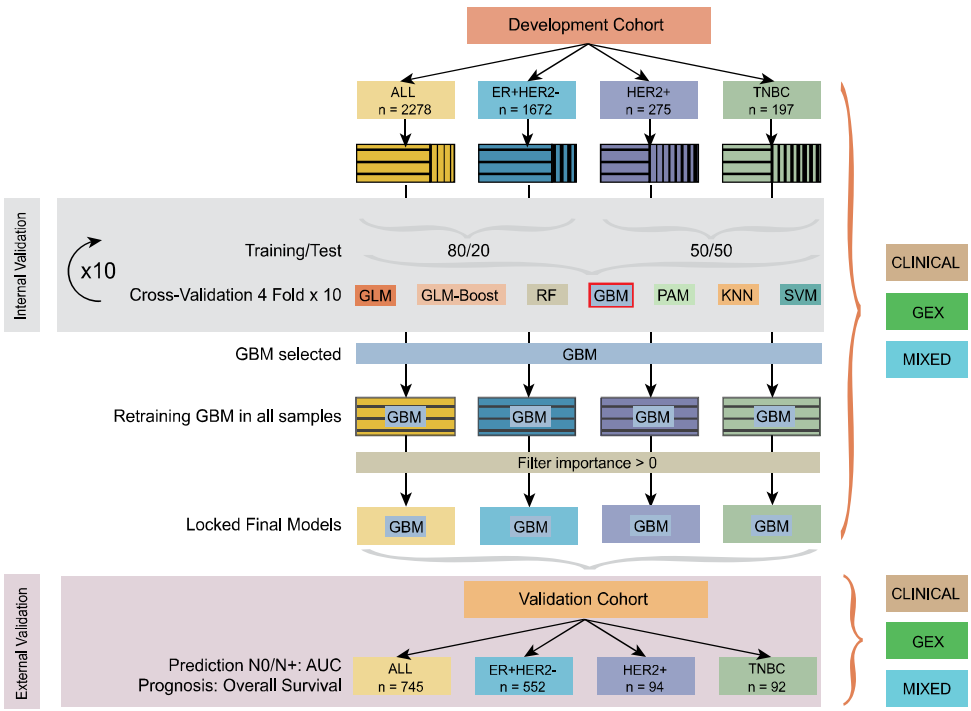


Figure 19. Acquisition of subtype-specific predictors of nodal status (N0/N+)
Overview of model selection, internal and external validation.

For each of the four evaluation groups (All, ER+HER2-, HER2+, TNBC), three classifiers were trained, one based on clinicopathological features alone (CLINICAL), one based on RNAseq data alone (GEX), and one with both clinicopathological parameters and RNAseq data (MIXED) (in a total of 7x4x3 separate models). The larger evaluation groups (All, ER+HER2-) were split into 80 percent training and 20 percent test groups, while the smaller evaluation groups (HER2+, TNBC) were split into 50 percent training and 50 percent test groups.

Internal validation was performed by ten iterations of four-fold cross-validations, repeated ten times. Mean area under the receiver operating characteristic (ROC) curve (AUC) identified generalized boosted models (GBM) with the highest predictive performance. The GBM model generates a relative importance score for each supplied feature (zero or positive). To create final locked models consisting of only significant features, the entire training procedure was re-iterated in a second loop for each evaluation group and model type (CLINICAL, GEX, MIXED), using only genes and clinicopathological features with a relative importance >0 from the first training run. This generated the final locked GBM models for validation in an independent dataset

RNAseq feature selection

For each evaluation group, RNAseq feature selection was performed for GEX and MIXED predictors in the training cohort. Of the 5000 most varying genes in each evaluation group, only genes remaining as differently expressed using a t-test between N0/N+ with < 10% Benjamin Hochberg corrected p-value threshold were selected for training. This approach, aimed to avoid inclusion of gene expression data with very little difference between N0/N+ disease, could thus yield different RNAseq features between the four evaluation groups.

Associations with transcriptional patterns

Unsupervised hierarchical clustering of the 500 most varying genes across tumors was performed in the validation cohort. Intrinsic breast cancer subtype classification according to PAM50[454] was performed using the AIMS R package. Calculation of biological metagene scores was performed as described by Fredlund et al[455].

For each evaluation group (All, ER+HER2-, HER2+, TNBC), functional pathway analyses displaying the biological processes associated with nodal metastasis predicted by the GEX and MIXED models was performed in the step preceding retraining as well as succeeding this step.

Logistic regression analysis

Variable selection

Logistic regression analysis is widely applied to estimate the effect of a variable on a dichotomous outcome. There are numerous variable selection algorithms available for multivariable regression analysis, e.g., forward selection, backward elimination, and more computationally intensive selection methods[456]. However, in Studies I-II, univariable logistic regression analysis was used to explore the unadjusted associations between variables and outcome and further inclusion of a variable in

the multivariable analysis was then based on both clinical relevance and statistical significance. The P-value cut-off point of 0.25 has been proposed in the literature, suggesting that traditional levels such as 0.05 could fail to identify significant variables[457,458]. If two or more variables were highly correlated, only one of them was retained in the final model to minimize multicollinearity. In Study I, Ki-67 status and histological grade were found to be strongly associated, as were the size of the largest nodal metastasis and the number of metastatic nodes. In the final multivariable logistic regression analysis, histological grade and size of metastatic deposits were retained. In Study II, Ki-67 status was incorporated into the St. Gallen surrogate definition of subtypes and was thus retained.

Effect on binary outcome

In a multivariable logistic regression analysis, the estimated regression coefficients take into consideration all included risk variables simultaneously. The exponentiated regression coefficients are odds ratios (ORs). OR yields the relative amount by which the odds of the outcome decreases (OR<1) or increases (OR>1) with one unit increase in value of a given risk variable[459]. OR can therefore be used to determine if a variable is a predictor for an outcome, and to compare the significance of different risk variables[378]. Logistic regression analysis was used to quantify the strength of associations between clinicopathological characteristics and different nodal status end-points in Studies II-III, and the association to abnormal axillary ultrasonography features in Study I. Mean ORs calculated as per Lippmann et al.[460] were applied in Study III.

Development of nomograms

The statistical description of a nomogram is a graphical illustration of a mathematical formula[378]. In Study II, regression coefficients from multivariable regression analyses were used to create three nomograms that predicted different extent of axillary metastatic involvement. A score factor proportional to the regression coefficient was assigned to each risk variable. The scaling of these was defined by arbitrarily appointing the score to 10 points for an extreme tumor size relative to the observed range. The user-contributed program nomolog.ado for Stata was used for nomogram development[461].

Statistical methodologies

Comparison between groups and inter-model agreement

In Study I, Mann-Whitney and χ^2 tests were used to compare the features of axillary metastatic involvement in patients with normal AUS features but a positive sentinel lymph node biopsy (AUS-SLNB+) with those having a positive ultrasonography-guided biopsy (AUS + FNAB+).

In study II, the distribution of clinicopathological variables and modes of detection across the five surrogate molecular subtypes of breast cancer was assessed by using the Pearson χ^2 test and Fisher's exact test for categorical variables, and Kruskal–Wallis test for continuous variables.

In Study III, the clinicopathological characteristics were evaluated across the ordered nodal classification outputs (N0, N1, N2) by Jonckheere-Terpstra test, χ^2 test for trend, Pearson χ^2 test, and Fisher's exact test, where appropriate. The predictive performances of ANN models and logistic regression models were compared using Wilcoxon signed-rank test.

In Study IV, Cohens's kappa assessed the inter-model agreement between the CLINICAL and MIXED models and between GEX and MIXED models for prediction of axillary nodal status (N0/N+).

Measurement of performance

Sensitivity and Specificity

The performance of a methodology or predictive model can be quantified by comparing the given results against a gold standard practice that verifies the true status of the patient.

In study I, sensitivity and specificity of axillary ultrasonography (AUS) and axillary ultrasonography-guided biopsy (AUS-FNAB) for preoperative nodal staging was assessed. Sensitivity quantifies the ability of a methodology to correctly identify the disease condition, while specificity is the ability of a test to correctly identify the subjects without the disease. Sensitivity and specificity of a test methodology are not affected by the prevalence of the disease, as these measurements are estimated from subgroups of individuals with and without the disease, respectively[462,463]. Nevertheless, sensitivity and specificity may be influenced by variances in the disease characteristics or characteristics of the patients, which was exemplified in Study I when sensitivity and specificity of AUS and AUS-FNAB were stratified by BMI.

Predictive values

The positive predictive value (PPV) and negative predictive value (NPV) are other fundamental indices of diagnostic accuracy. PPV is the probability that the disease is present given a positive test result. Correspondingly, the NPV is the probability that the disease condition is not present given a negative test result. PPV and NPV are affected by the prevalence of a disease condition[464].

In study III, a cut-off for classification of nodal-negativity was set corresponding to maximized NPV in the ANN model for disease-free axilla (N0). This cut-off was aimed to identify individuals with a very low probability of axillary nodal metastasis and would not be advocated SLNB by the model.

Table 4.
The two-by-two table is typically used to portray and assess the accuracy of a diagnostic test. Dichotomous test outcome is depicted in relation to the gold standard. Details of sensitivity, specificity, positive predictive value and negative predictive value measures are given.

Results of diagnostic tests	Results of gold standard test	
	Disease present	Disease absent
Positive	True positive (*a*)	False positive (*b*)
Negative	False negative (*c*)	True negative (*d*)

$$Sensitivity = \frac{True\ positives}{True\ positives + False\ negatives} = \frac{a}{a+c} \qquad Specificity = \frac{True\ negatives}{True\ negatives + False\ positives} = \frac{d}{d+b}$$

$$PPV = \frac{True\ positives}{True\ positives + False\ positives} = \frac{a}{a+b} \qquad NPV = \frac{True\ negatives}{True\ negatives + False\ negatives} = \frac{d}{d+c}$$

PPV, Positive predictive value; *NPV*, Negative predictive value

Discrimination

The receiver operating characteristic (ROC) curve analysis was introduced in the early 1950's and its application in medicine dates back to the early 1960's[465]. It is a popular measure for estimating classifier performance. The ROC curve is a plot of (1 – specificity) or false positive rate on the x-axis versus sensitivity or true positive rate on the y-axis across varying cut-offs[466]. The area under the curve (AUC) summarizes sensitivity and specificity over all possible cut-offs. Discrimination refers to the capability of the prediction model to make a distinction between patients with and without the end-point (e.g., N+ v*s*. N0). The maximum AUC of 1.0 corresponds to perfect discrimination whereas an AUC of 0.5 corresponds to a discrimination no better than chance[467,468].

In Studies II-IV, the discriminative ability of the nomograms and predictive models for different nodal status end-points was assessed by AUC values.

Calibration

While the discriminatory performance is generally the main focus in the evaluation of the performance of a predictive model, calibration is a pivotal aspect of model performance. Calibration describes the agreement between observed outcomes and predictions[469]. Previous publication have confirmed that the clinical utility of a predictive model is influenced by a model's calibration, the extent to which estimated risks correspond to observed event rates. The impact of miscalibration lessens, however, with increasing discriminative ability of a model[470].

In Study III, the Hosmer-Lemeshow (H-L) goodness-of-fit test was applied. It is one of the most commonly used goodness-of-fit tests for binary outcomes[459]. Generally, in a H-L test, samples are categorized into subgroups by decile of predicted probability or risk. A modified χ^2 test assesses if the expected and observed event rates in the subgroups are similar in the equal sized deciles of risk. The null hypothesis to be tested is that there is good agreement between the predicted and observed outcome and the model is fit. Thus, a p-value >0.05 suggests a calibrated model.

Validation

Validation is the process of determining the degree to which a predictive model could provide outcome predictions for new patients. Thus, validity addresses how a predictive model would perform in a similar population other than the one from which it was acquired[378].

Internal validation of a model determines its reproducibility (internal validity) in the setting where the development data is originated[378]. In Studies II-IV, internal validations were performed by means of bootstrapping and cross-validation. External validation refers to the generalizability or transportability of the prediction model. External validation evaluates if the model's predictions are adequate for clinical use in other plausible population(s)[396,471]. In Study IV, patients diagnosed with breast cancer during year 2011 constituted an independent validation cohort.

Bootstrap

In Study II, bootstrapping refers to a resampling procedure which is able to estimate the validity shrinkage of a predictive model. Bootstrap samples are created by random re-sampling with replacement from the original data set. Each bootstrap sample includes the same number of observations as the original sample. However, each patient may be excluded, included only once or several times. A prediction model is developed in the bootstrap sample and then validated in the original sample, and the difference in model performance indicates the optimism. To attain stable results, this procedure has to be repeated. Optimism is subtracted from the performance of the original model, and bootstrap validation (optimism-corrected or

bias-corrected) model performance is obtained[472,473]. Bootstrap resampling with 1000 replicates was used to estimate the accuracy of the prediction models in Study II.

Cross-validation

Cross-validation is a method that refers to the splitting of data into a separate training set (model development) and a validation or test set. The prediction model is tested in the validation set which was not part of model development[474]. In k-fold cross validation, the dataset is randomly split into k groups of equivalent size. One group is retained as the validation set, while the remaining, k-1 groups, are used for training/model development. Cross-validation is iterated k times (folds). Thus, each of the k groups is chosen as the validation set once. The performance estimation from the k-fold cross-validation is usually the average test values. The complete cross-validation procedure may be iterated multiple times to achieve more stable results, as applied in Studies III-IV[378].

Survival analysis

In Study IV, assessment of prognosis based on nodal status was performed in patients with ER+HER2- disease who received adjuvant endocrine therapy. Overall survival was the end-point and survival curves were estimated by the Kaplan-Meier method and the log-rank test. Hazard ratios (HR) and confidence intervals (CI) were estimated by Cox regression and displayed in Forest plots.

Analysis software

Statistical computations were performed in SPSS Statistics for Windows version 22.0 and 24.0 (IBM, Armonk, New York, USA); Stata version 14.1 (StataCorp, College Station, Texas, USA); R using the Caret package and the coxph R function and custom made software written in C (gcc version 4.8.5) and Perl (version 5.18.2).

Methodological considerations

Study design, source of data and quality of data

The accuracy of a prediction model is dependent on the quality of the input data. Retrospective cohort studies will more likely lead to biased conclusions than studies with prospective design since they are constructed from records that have previously been gathered. Accordingly, there might be bias related to data quality, data assembly and data entry. Specifically, selection bias could occur related to missing data for potential confounders. Further limitations include not accurately identifying patients and related variables in retrospect. Single center studies are restrained by

their sample size, which is a crucial problem in prediction studies and could jeopardize the generalizability of the prediction model. Thus, strategies for predictive validity must be applied. In comparison with a randomized control trial, a cohort study usually has wider inclusion criteria and may be more generalizable to clinical implications[378].

There are approaches that can help improve the accuracy of the data used in the analysis. For example, a recent meta-analysis reported that neoadjuvant chemotherapy results in axillary pathologic complete response in 30-40% of clinically node-positive[475]. By excluding patients scheduled for neoadjuvant therapy in Studies I-IV, the confounding influence on axillary nodal status was diminished.

The cohorts for Studies I-III were retrieved from a breast cancer histopathology database designed for prospective data collection and quality control. All tumor features and nodal metastatic deposits were analyzed according to validated protocols and the data were managed by a single breast pathologist, which helped to improve the consistency of analyses thereby minimizing inaccuracies. The database for Studies I-III was primarily designed for quality assurance but it provided comprehensive histopathological characteristics that enabled detailed analysis on tumor and nodal metastatic features as predictors. Nevertheless, missing data for certain central variables (e.g., LVI) must be accounted for in the interpretation of the results. Accordingly, the incompleteness of data for each predictor variables is presented in the studies.

Although Study IV is based on a prospective, observational cohort design, the clinicopathological characteristics and survival data were retrospectively obtained from registries. Thus, the study suffers from some of the limitations as retrospective studies described previously. Clinicopathological variables were obtained from the Swedish National Quality Registry for Breast Cancer which includes prospective data on demographics, disease characteristics and treatments. While the registry coverage is almost 100%[32], there are missing data for specific variables which could otherwise be included in predictor training or be used for defining evaluation groups (e.g., Ki-67 and size of the nodal metastatic deposit). Likewise, no predefined assessments were made for the prognostic analysis in Study IV and the outcome analysis was limited to overall survival. In the interpretation of the outcome, the median follow-up time of 5.8 years for patients with ER+HER2- was relatively short. The other principal quality aspect for data inclusion in Study IV is related to RNAseq. Tissue sample treatment and RNAseq was performed at a central laboratory, which certifies real-time quality controlled RNAseq data.

Variable and end-point definition

Precise and standardized definitions of the predictor variables and outcome end-points are fundamental in the interpretation and comparison of predictor models. In

this thesis, axillary node positivity was defined as the presence of micrometastases or macrometastases. For studies on nodal status prediction, it is important to recognize that the nodal status can be influenced by histological techniques employed (e.g., serial sectioning, immunohistochemistry or molecular techniques). Likewise, for assessing the predictive power of a variable on nodal status outcome, differences in variable definitions should be acknowledged (e.g., differences in cut-off levels for hormone receptor status and Ki-67).

Effective sample size

The frequency of the defined end-point, rather than the overall number of patients included in a study cohort, verifies the effective sample size[378]. This is exemplified in Study I by the rather small samples of patients presenting with malignant axillary ultrasonographic features and malignant FNAB findings (AUS+FNAB+) as well as the limited numbers of patients with heavy-burden axillary metastatic disease in Studies II-III. Likewise, there is a limited number of HER2+ and TNBC tumors in Study IV despite analyzing > 3000 cases.

Data classification and variable transformation

Conversion of continuous variables to categorical variables by organizing values may cause arbitrariness and relevant loss of information[476]. However, variables were categorization to identify specific characteristics of concepts to be measured (e.g., obesity and molecular subgroups) and for descriptive purposes. Data classification has been performed in accordance with standardized and accepted definitions.

Transformations of variables can be used to present data on a different scale during model development and the type of transformation defines how the scale of the untransformed variable will be modified. Variable transformations are commonly applied to increase the compatibility of the data with the assumptions of the model statistics, for normalization, linearization or stabilization of variance. Moreover, variable transformation may help to simplify data management and improve the interpretability of the outcome. However, the fact that variable transformation could alter the associations that existed among the original variables should not be ignored. It is also possible that an improvement in one modeling assumption by variable transformation could cause intrusion of other assumption essential to the model[477,478].

Missing data

Several techniques were applied to handle missing values in the development of prediction models in Studies II-IV. Study II included only patients with complete data on ER, PR, HER2 and Ki-67 status, as defined by the 2013 St. Gallen surrogate definition of breast cancer subtypes. In Study III, missing data were handled by multiple random imputation and in Study IV, missing values were recoded with a

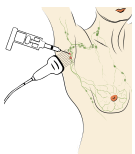
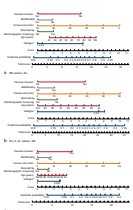
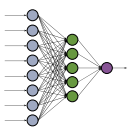
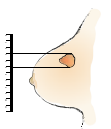
specific value for each variable and the clinicopathological data did not contain any missing entries.

In prediction research, the issue of incomplete data fields is a common challenge. Incomplete data could render inefficiency in the analyses of research end-points (e.g., when applying complete case analysis and discarding subjects with partially missing data) and impede the understanding of the outcome when the number of subjects diverge between analyses (e.g., when applying available case analysis). Furthermore, there are concerns of bias related to systematic differences between patients with complete data and patients with missing data. Previous studies have suggested the ANN models are more tolerant to missing data than risk predicting models based on logistic regression analysis[460,479]. Application of imputation techniques may be superior to complete case analysis in the development of prediction models, especially for increasing the power to detect outcome effects and also to provide comparability of outcome between analyses[378].

Multiple hypothesis testing

If numerous null hypotheses were tested at the same time in a dataset with many potential predictor variables (e.g., those produced by high-throughput research), the probability of rejecting the null hypothesis erroneously would be greater than the pre-specified level of significance[480]. Simultaneous inference procedures focus on adjusting for multiplicity, and controlling the Type I error rate while preserving power of the individual tests[395]. In Study IV, the Benjamini-Hochberg procedure[481] was applied in the feature selection process (top 5000 varying genes, t-test histopathological N0/N+ and <10% Benjamin Hochberg corrected p-value). Nevertheless, there are several methodologies for multiplicity issues, each with merits and limitations that could influence the final outcome[482]

Strengths and limitations

Study	Strenghts	Limitations
I 	Data from a prospectively maintained database. No influence of confounding neoadjuvant treatment. Detailed clinicopathological data. Tumor and nodal characteristics analyzed according to validated protocols. All histopathological data were managed by an experienced breast pathologist. Inclusion of predictors for multivariable logistic regression were based on both clinical and statistical significance. Multivariable logistic analysis adjusted for other relevant clinicopathological factors.	Operator dependent performance of AUS. Limited number of AUS-FNAB cases. No standardized classification for aberrant AUS findings in the literature. Evaluation of newer AUS techniques e.g., elastography and contrast-enhanced ultrasound (CEUS) was not performed.
II 	As in Study I, and with the addition of: Complete records on ER, PR, HER2+ and Ki-67 for St. Gallen surrogate breast cancer subtype classification. Exclusion of cases with preoperatively verified nodal metastasis. Exclusion of cases with outlier tumor size. The prediction tools are not dependent on SLNB characteristics. Prediction tools are presented in a user-friendly graphical format. Internal validation performed.	High number of missing data on LVI. Histopathological data from postoperative pathology reports used for the model development. Preoperative utility dependent on the reliability of radiological assessments for tumor size measurements. Limited number of cases with heavy-burden nodal metastasis. Nomograms not externally validated.
III 	As in Study I, and with the addition of: Largest study to date to present ANN-based models for prediction of nodal metastatic burden in a population-based, contemporary cohort. Exclusion of clinically node-positive cases. Internal validation performed. Model calibration was assessed. Dropout technique prevented overfitting. Clinical relevant cut-offs was evaluated to suggest the utility in reducing unnecessary SLNB.	As in Study II, and with the addition of: The “black box” nature of ANN models limits the capability to clearly identify possible causal relationship between predictors and nodal status outcome. Lack of user-friendly interface. Not prospectively validated in an external cohort.
IV  	Largest cohort to date to evaluate RNAseq as predictors of nodal metastasis, alone or in combination with clinicopathological factors. Subtype specific predictors. Prospectively collected data. Population-based, multicenter cohort. Protocol to ensure high quality RNAseq. Several machine learning models and feature-selection approaches applied. External validation performed.	Clinicopathological data from registries. High number of missing data on multifocality and LVI. St. gallen subtypes not possible to obtain due to missing data on Ki-67 The end-points defined from registry data did not enable more refined classifications. Limited number of HER2+ and TNBC tumors. Short follow-up time (OS).

Results

The results from the Studies I-IV are summarized separately in this section.

Key findings from Study I (Axillary ultrasonography and ultrasonography-guided biopsy) and Studies II-IV (Predicting the risk of nodal metastasis and the metastatic burden) are further presented and discussed in the next section (Discussion) in the context of current evidence on axillary treatment, and with special focus on clinical utility.

Study I - Axillary ultrasonography and ultrasonography-guided biopsy

Axillary ultrasonography (AUS) was performed in 473 patients. Abnormal nodal features was identified in 55 patients and fine-needle aspiration biopsy (FNAB) was performed in 45 of these. Malignant cytology (C5) was found in 24 patients and these patients proceeded directly to ALND. For 125 patients, a normal AUS was followed by SLNB which revealed metastatic deposits.

Low sensitivity of axillary ultrasonography

The sensitivity of AUS for the detection of metastatic disease was low. AUS alone had an overall sensitivity of 40/175 (23%). However, the performance improved when combined with ultrasonography-guided biopsy, revealing a sensitivity of 24/33 (73%) and the specificity increased from 95% to 100%.

The accuracy is dependent on the metastatic burden

Axillary metastatic burden, indicated by the nodal metastatic size in millimeters and number of involved ALNs, was the most important predictor of an abnormal AUS results. This suggests that AUS is unreliable in patients with low metastatic burden. For each metastatic lymph node, the prediction accuracy of AUS was improved by 20%, while for each mm of metastatic deposit, the accuracy improved by 11%.

Clinicopathological factors affecting the performance

Contrary to what might be expected, the results indicated that the performance of AUS alone was improved in obese patients, with a 2.5-fold increase in sensitivity compared with those with a BMI <30 kg/m^2. There were no BMI-related differences observed in the performances of AUS-guided FNAB. Histological grade was found to add independent information on the accuracy of ultrasonography performance and patients with HER2+ tumors had higher rates of AUS abnormalities.

Higher axillary metastatic load with suspicious ultrasonography findings

The axillary metastatic burden in patients diagnosed with nodal metastasis by ultrasonography-guided biopsy (AUS+FNAB+) was compared with that in patients with normal ultrasonography findings but a metastatic sentinel node (AUS-SLNB+). The total metastatic burden was higher in the AUS+FNAB+ group compared with the AUS-SLNB+ group; there were higher incidence of N2 disease, and a greater median metastatic deposit size (15 *versus* 3 mm). High rate of micro-metastases (39%) was found in the AUS-SLNB+ group, whereas no micro-metastases were identified in the AUS+FNAB+ group, supporting the observation that ultrasonography-guided biopsy of the axilla could not detect minor tumor deposits in the axillary lymph nodes (Table 5).

Table 5.
Nodal status in patients with normal axillary ultrasonography features but a positive sentinel lymph node biopsy (AUS- SLNB+) in comparison with patients presenting with positive ultrasonography-guided biopsy (AUS+ FNAB+).

	AUS- SLNB+	AUS+ FNAB+	*p*
Metastatic nodal disease, n	125	24	
No. examined nodes, median (range)	15 (2-38)	16 (4-32)	1.0[a]
No. metastatic nodes, median (range)	1 (1-16)	4 (1-30)	<0.001[a]
1-3 metastatic nodes, n (%)	107 (86)	11 (46)	<0.001[b]
>3 metastatic nodes, n (%)	18 (14)	13 (54)	<0.001[b]
Size metastatic deposit, median (range)	3 (0.22-32)	15 (4.5-50)	<0.001[a]
Micrometastases, n (%)	49 (39)	0 (0)	<0.001[b]
Macrometastases, n (%)	76 (61)	24 (100)	<0.001[b]

[a] Mann-Whitney test
[b] χ^2-test

Study II - Nomograms for prediction of axillary nodal status

The study cohort consisted of 692 patients. The nodal status distribution was: disease-free axilla: 444 (64%); limited axillary nodal metastasis (1-2 positive lymph nodes): 170 (25%); and heavy-burden axillary nodal metastasis (≥ 3 positive lymph nodes): 78 (11%).

According to the 2013 St. Gallen surrogate definition of breast cancer subtypes, 372 tumors were identified as Luminal A-like, 198 as Luminal B-like HER2-, 64 as Luminal B-like HER2+, 17 as HER2+ non-luminal and 41 as triple-negative. The clinicopathological factors that varied across the subtypes were: age, mode of detection, tumor size, histological type, histological grade, LVI and the amount of axillary nodal metastasis (N0 *versus* N+).

Predictors of nodal status - multivariable logistic regression analysis

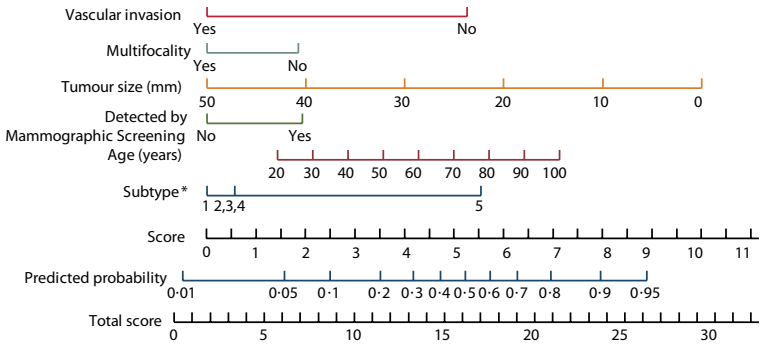
Tumor size was the single most significant factor associated with nodal status. The odds of having 1-2 metastatic lymph nodes and ≥ 3 metastatic lymph nodes were increased by 5 and 8 per cent, respectively, for each millimeter increase in tumor size. Increasing age, detection of the tumor by mammographic screening (in contrast to symptomatic presentation), and the absence of multifocality and LVI were found to be positively associated with benign lymph nodes in patients with T1-T2 breast cancer. The odds of having disease-free axilla was more than five times higher for the triple-negative subtype than for the Luminal A-like subtype (Table 6).

Nomograms predicting the extent of nodal metastatic involvement

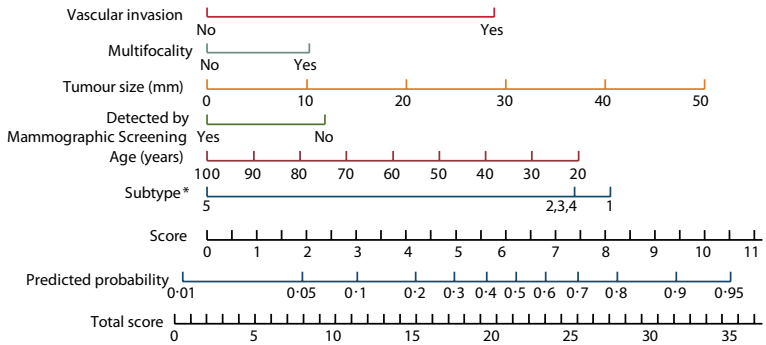
The results from the multivariable regression analyses were used to develop three nomograms to predict disease-free axilla N0, limited axillary nodal metastasis N+(1-2 positive lymph nodes), and heavy-burden axillary nodal metastasis N+(≥ 3 positive lymph nodes) (Figure 20).

Predictive performance and internal validation

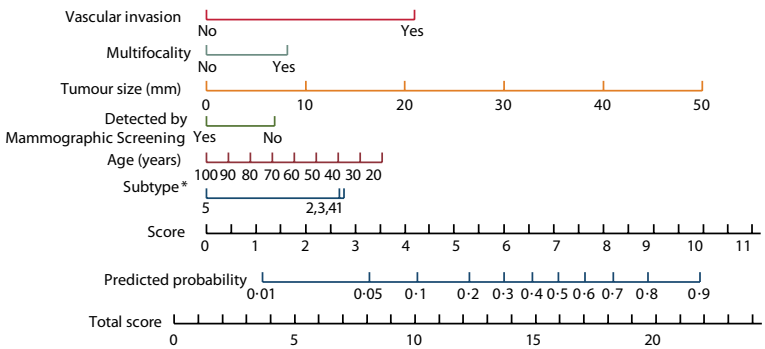
The AUC to distinguish disease-free axilla, N0 *versus* N+, was 0.74 (95% CI 0.70-079). Using 1000 resampled bootstrap data sets, the bias-corrected AUC was 0.74 with a decrease (–0.009) in discriminative ability. The AUC to classify limited axillary nodal metastasis, N+(1–2) *versus* N0, was 0.70 (95% CI 0.65-0.75) with a bias-corrected AUC of 0.69 (–0.013). AUC to distinguish heavy-burden axillary nodal metastasis, N+(≥ 3) *versus* N0 and N+(1–2), was 0.81 (95% CI 0.75-0.86) with a bias-corrected AUC of 0.79 (–0.013).



a N0 versus N+



b N+(1–2) versus N0



c N+(≥ 3) versus N0 and N +(1–2)

Figure 20. Nomograms predicting the extent of axillary nodal disease
a Disease-free axilla (N0) versus any nodal metastasis (N+).
b Low-volume axillary disease involving one or two nodes (N+(1–2)) versus N0.
c High-volume axillary disease involving at least three nodes (N+(≥3)) versus N0 and N+(1–2).
The total score for each patient is assigned by drawing a vertical line from the appropriate point for each predictor down to the score scale, and summing these scores. To obtain the predicted probability of a specific nodal status, a vertical line is drawn from the total score scale up to the predicted probability scale in the lower part of the nomogram.
* Subtypes: 1, Luminal A-like; 2, Luminal B-like (LumB)/human epidermal growth factor receptor 2 (HER2)-negative; 3, LumB/HER2-positive; 4, HER2-positive/non-luminal; 5, triple-negative
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Table 6.
Multivariable logistic regression for prediction of axillary nodal status

	Axillary nodal status					
	N0 vs. N+ (n=598)		N+(1-2) vs. N0 (n=535)		N+(≥3) vs. N0 and N+(1-2) (n=598)	
	Odds ratio	*p*	Odds ratio	*p*	Odds ratio	*p*
Subtype		0.031		0.063		0.470
LumA	1.00		1.00		1.00	
LumB/HER2–	1.18 (0.76-1.84)		0.87 (0.53-1.41)		0.84 (0.42-1.67)	
LumB/HER2+	1.11 (0.56-2.21)		0.87 (0.40-1.88)		1.43 (0.59-3.48)	
HER2+ / non-luminal	1.48 (0.40-5.44)		0.66 (0.16-2.73)		0.79 (0.13-5.03)	
Triple-negative	5.06 (1.89-13.50)		0.17 (0.05-0.54)		0.36 (0.09-1.46)	
Age (per year)	1.02 (1.00-1.04)	0.013	0.98 (0.96-1.00)	0.023	0.99 (0.96-1.01)	0.227
Mode of detection		0.006		0.021		0.079
Symptomatic	1.00		1.00		1.00	
Mammographic screening	1.75 (1.18-2.61)		0.60 (0.39-0.93)		0.58 (0.32-1.06)	
Tumour size (per mm)	0.94 (0.92-0.97)	<0.001	1.05 (1.02-1.07)	<0.001	1.08 (1.04-1.11)	<0.001
Multifocality		0.015		0.064		0.053
Yes	1.00		1.00		1.00	
No	1.72 (1.11-2.65)		0.64 (0.39-1.03)		0.54 (0.29-1.00)	
Vascular invasion		<0.001		<0.001		<0.001
Yes	1.00		1.00		1.00	
No	4.67 (2.70-8.09)		0.28 (0.15-0.51)		0.21 (0.11-0.39)	

Values in parentheses are 95 per cent confidence intervals. *N0*, lymph node-negative; *N+*, any lymph node metastasis; *N+(1–2)*, lymph node metastasis involving one or two nodes; *N+(≥3)*, lymph node metastasis involving at least three nodes; *LumA*, Luminal A-like; *LumB*, Luminal B-like; *HER2*, human epidermal growth factor receptor 2.

Number of metastatic lymph nodes in relation to tumor size

The increase in tumor size was found to be less often associated with metastatic nodal involvement in the TNBC subtype than in other non-TNBC subtypes (Table 7 and Figure 21).

Table 7.
Univariable logistic regression models for axillary lymph node metastasis with tumour size in four categories as the only co-variable among patients with triple-negative disease and all other patients

	No. of patients			
Tumor size (mm)	N0	N+	Odds ratio	*p*
Non-triple-negative (n=651)				<0.001
1–10	139	30	1.00 (reference)	
11–20	192	107	2.58 (1.63-4.09)	<0.001
21–30	65	73	5.20 (3.10-8.73)	<0.001
>30	15	30	9.27 (4.45-19.32)	<0.001
Triple-negative (n=41)				0.342
1–10	6	1	1.00 (reference)	
11–20	18	2	0.67 (0.05-8.73)	0.757
21–30	6	3	3.00 (0.24-37.67)	0.395
>30	3	2	4.00 (0.25-63.95)	0.327

Values in parentheses are 95 per cent confidence intervals. *N0*, lymph node-negative; *N+*, any lymph node metastasis.

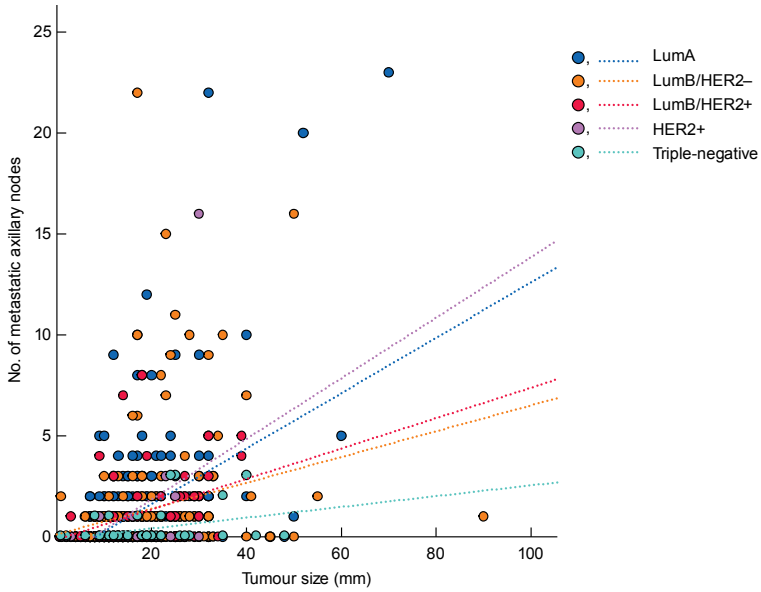

Figure 21.
Scatterplot of number of metastatic axillary nodes versus tumor size stratified by breast cancer surrogate molecular subtype. Trend lines (dotted) are shown only to facilitate comparison among the five molecular subtypes. *LumA*, Luminal A-like; *LumB*, Luminal B-like; *HER2*, human epidermal growth factor receptor 2
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Study III - Artificial neural network models for prediction of axillary nodal status

The study cohort consisted of 800 patients with clinically node-negative breast cancer. The nodal status distribution was: disease-free axilla, N0: 514 (64%); limited axillary nodal metastasis (1-3 positive lymph nodes, N1): 232 (29%); and heavy-burden axillary nodal metastasis (≥ 4 positive lymph nodes, N2): 54 (7%).

Predictors of nodal status in artificial neural network models

The discriminatory effect of a specific variable in ANN models cannot not be stated in terms of straightforward coefficients. However, mean odds ratios and sensitivity analysis facilitate the interpretation of the relationship between a variable and the nodal status end-point. The significance of selected variables in each nodal status end-point was assessed by randomizing a variable across the evaluation cohort. The variable corresponding to the greatest decrease of discriminatory performance will be the most important and given the importance value of 1. All other variables are given a position in this list based on their decrease in performance when randomized.

Table 8. Predictive clinicopathological variables in the ANN models for each of the nodal status end-point

N0 vs. N+ (n=800) Predictors	Rank[a]	Odds ratio[b]
Tumor size, per mm	100.00	0.950 (0.917-0.984)
Vascular invasion, present vs. absent	40.94	0.409 (0.201-0.681)
Multifocality, present vs. absent	14.61	0.670 (0.452-0.910)
ER status, positive vs. negative	10.49	0.618 (0.312-1.110)
Histological type	9.98	
Ductal		1 [reference]
Lobular		1.092 (0.692-1.688)
Other		2.033 (1.112-3.751)
PR status, positive vs. negative	9.60	0.678 (0.443-0.962)
Mode of detection, mammographic screening vs. symptomatic presentation	7.97	1.310 (0.987-1.705)
Age, per year	6.76	1.010 (0.997-1.024)
Tumor localization in the breast	6.47	
Upper outer quadrant		1 [reference]
Central		1.137 (0.592-2.099)
Upper inner quadrant		1.323 (0.922-1.889)
Lower inner quadrant		1.112 (0.500-2.034)
Lower outer quadrant		0.680 (0.383-1.039)
Ki67, percentage	5.07	0.996 (0.981-1.009)
N1 vs. N0 (n=746) **Predictors**	**Rank[a]**	**Odds ratio[b]**
Tumor size, per mm	100.0	1.050 (1.016-1.087)
Vascular invasion, present vs. absent	46.15	2.492 (1.440-4.376)
Multifocality, present vs. absent	16.47	1.527 (1.101-2.180)
PR status, positive vs. negative	14.36	1.613 (1.058-2.409)
Histological type	10.55	
Ductal		1 [reference]
Lobular		0.785 (0.466-1.131)
Other		0.491 (0.242-0.872)
ER status, positive vs. negative	9.04	1.657 (0.813-2.895)
Age, per year	6.95	0.992 (0.978-1.003)
Tumor localization in the breast	6.03	
Upper outer quadrant		1 [reference]
Central		0.805 (0.324-1.415)
Upper inner quadrant		0.741 (0.510-1.080)
Lower inner quadrant		0.974 (0.476-1.759)
Lower outer quadrant		1.462 (0.915-2.369)
Ki67, percentage	5.07	1.002 (0.992-1.014)
Menopausal status, postmenopause vs. premenopause	4.88	0.783 (0.504-1.065)
N2 vs. N0 and N1 (n=800) **Predictors**	**Rank[a]**	**Odds ratio[b]**
Tumor size, per mm	100.0	1.039 (1.020-1.054)
Vascular invasion, present vs. Absent	36.71	1.805 (1.345-2.451)
ER status, positive vs. negative	13.25	1.777 (1.357-2.873)
Histological type	4.55	
Ductal		1 [reference]
Lobular		1.658 (1.252-2.083)
Other		0.910 (0.401-1.194)
Tumor localization in the breast	3.48	
Upper outer quadrant		1 [reference]
Central		1.208 (0.739-2.033)
Upper inner quadrant		1.012 (0.778-1.270)
Lower inner quadrant		1.077 (0.684-1.410)
Lower outer quadrant		1.544 (1.134-2.021)
Multifocality, present vs. absent	2.32	1.219 (1.033-1.338)

N0, Lymph node negative; *N+*, Any lymph node metastasis; *N1*, Lymph node metastasis involving 1-3 nodes; *N2*, Lymph node metastasis involving at least 4 nodes; [a] Sensitivity analysis, linearly scaled into percentage; [b] Mean odds ratio, values enclosed by parentheses represent 90% central range defined by the 5th and 95th percentiles

Tumor size and LVI remained the top two variables most strongly associated with any of the nodal status end-point. Other variables of significance (age, multifocality, histological type, ER status, PR status, Ki-67 values, mode of detection, and tumor localization in the breast) varied in rank of association with disease-free axilla, limited axillary nodal metastasis and heavy-burden axillary nodal metastasis. Non-linear dynamic associations were displayed between the predictors and each nodal status end-point (Table 8).

Predictive performance and internal validation

Internally validated AUC to distinguish disease-free axilla (N0 *versus* N+) was 0.74 (95% CI 0.72-0.76). AUC to classify limited axillary nodal metastasis (N1 *versus* N0) was 0.71 (95% CI 0.69-0.72). To distinguish heavy-burden nodal metastasis (N2 *versus* N0 and N1), AUC of 0.75 (95% CI 0.73-0.77) was obtained.

For each nodal status end-point, ANN models showed better discriminatory performance than matching multivariable logistic regression models.

Possible sentinel lymph node biopsy reduction

The possible clinical benefit of using the ANN model stratification in detecting patients least likely to benefit from SLNB was assessed. A cut-off value according to maximum negative predictive value or false-negative rate of 5–10% in the model to discriminate N0 would yield a SLNB reduction rate of 8–27% (Table 9).

Table 9.
SLNB reduction rates using the ANN model to predict disease-free axilla. Possible SLNB reduction rate corresponding to cut-offs at maximum negative predictive value, false negative rate 5% and 10%, respectively.

N0 vs. N+ (n=800)				
Cut-off Max NPV 0.95	TP	TN	FP	FN
No.	283	57	457	3
SLNB Reduction Rate	(TN+FN) / (TP +TN +FP +FN) = 8%			
False Negative Rate	FN / (TP+FN) = 1%			
Cut-off NPV 0.90	TP	TN	FP	FN
No.	272	128	386	14
SLNB Reduction Rate	(TN+FN) / (TP +TN +FP +FN) = 18%			
False Negative Rate	FN / (TP+FN) = 5%			
Cut-off NPV 0.87	TP	TN	FP	FN
No.	258	190	324	28
SLNB Reduction Rate	(TN+FN) / (TP +TN +FP +FN) = 27%			
False Negative Rate	FN / (TP+FN) = 10%			

N0, Lymph node negative; *N+*, Any lymph node metastasis; *SLNB*, Sentinel lymph node biopsy; *NPV*, Negative predictive value; *TP*, True positive; *TN*, True negative; *FP*, False positive; *FN*, False negative

Study IV - Clinicopathological and gene expression models for prediction of axillary nodal status

The study cohort consisted of 2278 patients in the development cohort and 745 patients in the external validation cohort. Similar clinicopathological characteristics for all patients in the catchment region and among those in the development and validation cohorts were observed, which reinforce the population-based nature of the study cohorts.

Tumors were classified into surrogate molecular subtypes based on the pathological report of ER, PR, and HER2 status. Within the development cohort, 1672 tumors were classified as ER+HER2-, 275 as HER2+ and 197 as TNBC. In the independent validation cohort, 552 tumors were identified as ER+HER-, 94 as HER2+ and 92 as TNBC

Axillary lymph node metastasis, N+, was found in 36% and 34% of the patients in the development and validation cohorts, respectively.

Predictors of nodal status and related biological processes

Predictors from the models including clinicopathological features alone (CLINICAL), RNAseq data alone (GEX), and mix of clinicopathological features and RNAseq data (MIXED) were evaluated.

Tumor size, LVI, age, and multifocality were the most significant variables associated with axillary nodal metastasis among the clinicopathological predictors (CLINICAL). When gene expression data were included (MIXED), tumor size and LVI remained the top two variables associated with nodal disease in the validation cohort for ER+HER2- cases and HER2+ cases. For the TNBC cases, however, five genes ranked higher than these variables.

Higher proportions of the Luminal B intrinsic features and proliferation-related genes were observed in ER+HER2- and HER2+ tumors with predicted node-positive disease. Low expression of basal-like markers was detected for TNBC tumors with predicted nodal metastasis (Figure 22).

Predictive performance, external validation and prognostic assessment

Of seven machine-learning models, Generalized Boosted Models (GBM) was identified with the highest predictive performance to distinguish N0/N+ disease.

Figure 23a summarizes performances of the GBM-models for prediction of nodal metastasis in the development cohort, with the MIXED predictors obtaining highest performance in all evaluation groups.

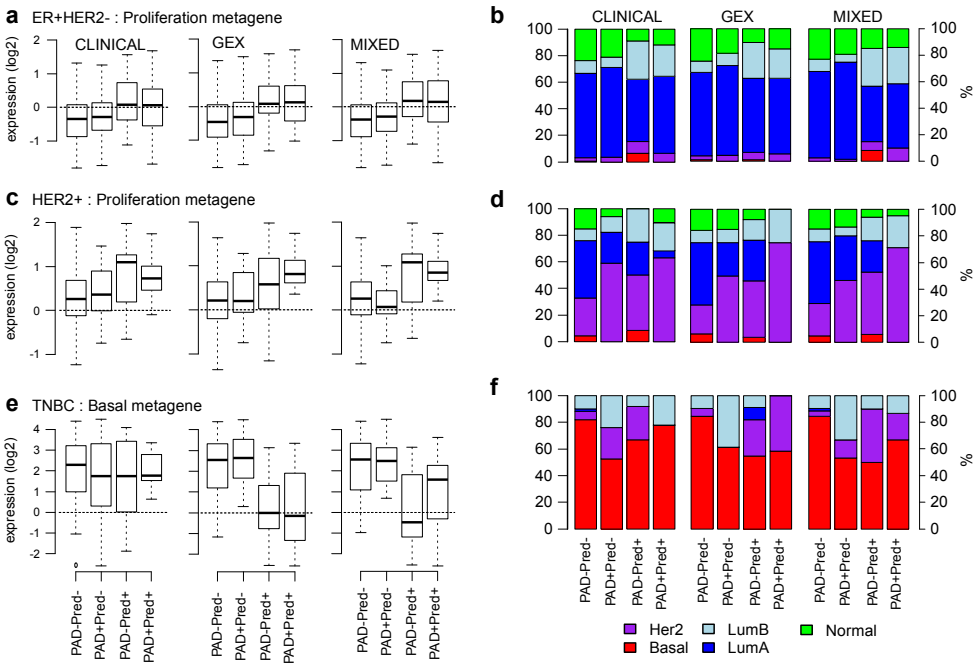

Figure 22. Associations of derived nodal predictors with transcriptional patterns and subtypes
Four nodal status subgroups were evaluated, stratified by combinations of pathological nodal status, N0/N+ (PAD-/PAD+) and model-predicted axillary status, N0/N+ (Pred-/Pred+) in the validation cohort. Expression of the checkpoint proliferation metagene, and PAM50 classification within a-b ER+HER2-, and c-d HER2+ evaluation groups. e Expression of a basal metagene, and f distribution of PAM50 subtypes within the TNBC evaluation group. In a, c, and e, boxplots show expression of metagenes for the CLINICAL (left), GEX (center), and MIXED (right) models.

PAD, pathology-defined nodal status; *Pred*, model-predicted nodal status; *CLINICAL*, predictor based on clinicopathological features alone; *GEX*, predictor based on RNA sequencing (RNAseq) data alone; *MIXED*, predictor based on both clinicopathological parameters and RNAseq data.

In the overall validation cohort (n=745), the MIXED predictors achieved the highest discriminative performance, AUC 0.72 (95% CI 0.68-0.76). However, the CLINICAL predictors showed comparable discriminatory performance of AUC 0.71 (95% CI 0.67-0.75). Thus, the addition of gene expression data to routine clinicopathological variables did not show a clear superiority in predicting nodal status in the evaluation groups defined by surrogate molecular subtypes (Figure 23b).

For patients with ER+HER2- tumors receiving adjuvant endocrine therapy, a worse prognosis (OS) was observed if node-positive disease was predicted by the models compared to a prediction of a disease-free axilla, irrespective of the histopathological nodal status. Patients with both histopathological and model-predicted node-positive disease (PAD+Pred+) had the worst prognosis. The prognostic importance of the PAD+Pred+ was significant in a Cox multi-variable

analysis based on the GEX predictor when adjusted for tumor size and age (HR 2.13, CI 1.06-4.26, p=0.03), but was not independent of histological grade.

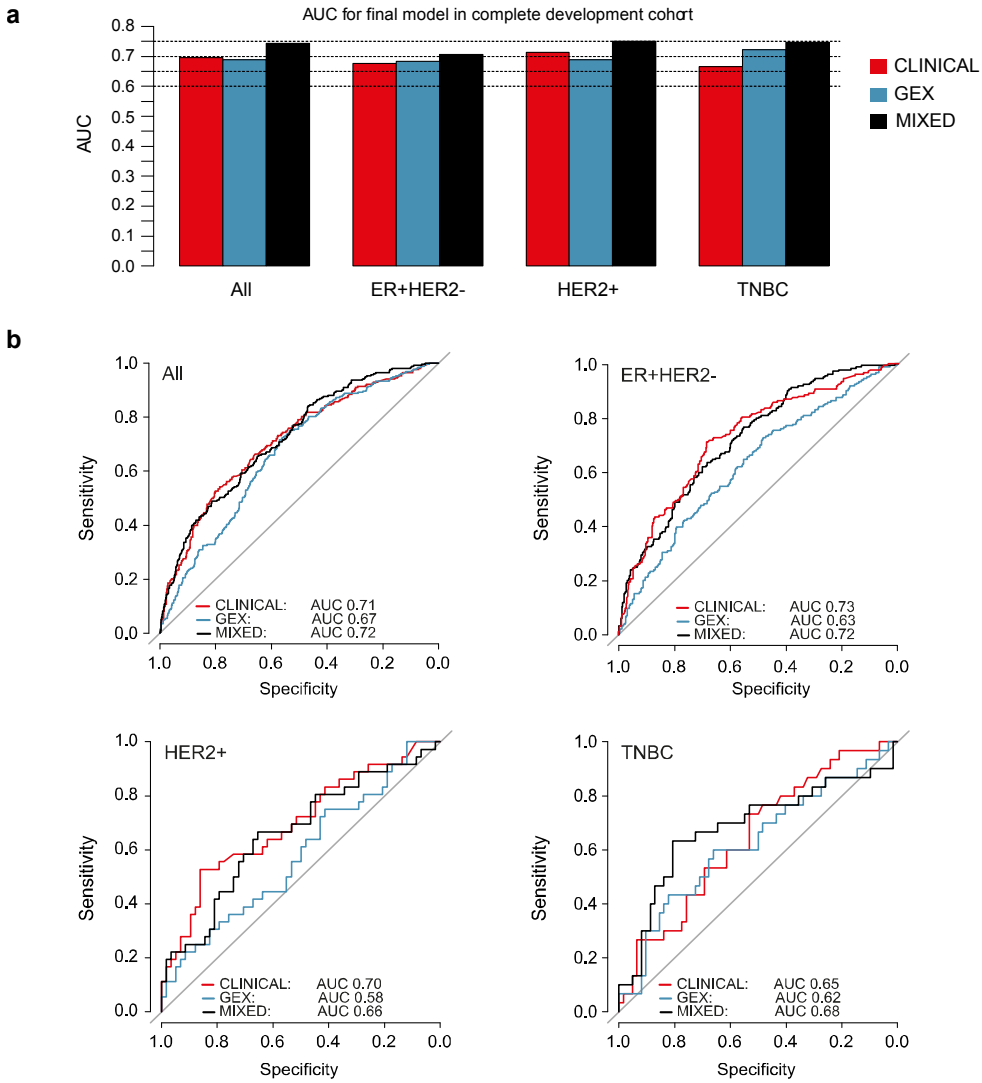

Figure 23. Performance in predicting N0/N+
a Performance of the three GBM-based predictor models in predicting nodal metastasis presented as area under the receiver operating characteristic (ROC) curve (AUC) in the entire development cohort and stratified in subgroups. b AUC curves estimating the predictive performance in the independent validation cohort for all tumors and subgroups. *CLINICAL*, predictor based on clinicopathological features alone; *GEX*, predictor based on RNA sequencing (RNAseq) data alone; *MIXED*, predictor based on both clinicopathological parameters and RNAseq data.

“Everything should be made as simple as possible, but not simpler”

-paraphrase of Albert Einstein

Discussion

Axillary ultrasonography and ultrasonography-guided biopsy (Study I)

Ability to detect metastasis

The first reports to introduce and evaluate the technique of ultrasonography-guided biopsy date back more than 20 years[419]. To date, preoperative axillary staging by imaging has not been able to replace surgical staging due to inferior sensitivity compared with SLNB. Study I indicated that the sensitivity of AUS for the detection of metastatic lymph nodes was low. While AUS alone yields fair accuracy, particularly in detecting limited metastatic burden, the results indicated that sensitivity and specificity to detect nodal metastasis could be enhanced when combined with ultrasonography-guided biopsy. Two contemporary meta-analyses revealed the pooled sensitivity of AUS to be 50–55%[418,483]. For ultrasonography-guided biopsy, an updated review, including data from 2805 breast cancer patients, revealed pooled sensitivity of 80% and specificity of 98%, a median PPV of 100%, and an NPV of 67%[484]. Our result on FNR is in accordance with the findings of a systematic review, which concluded that as many as one in four patients with negative ultrasonography-guided biopsy of the axilla presented with metastatic sentinel nodes[418].

In the literature, there is great diversity for reported sensitivity and FNR[418,483] related to AUS. One explanation for this is the difference in the prevalence of axillary metastasis among the study populations, which has been reported to range between 25% and 58%[485]. Other reasons are patient selection, exclusion criteria, and disparities in the specific definitions to classify a lymph node as suspicious on AUS. The main recruitment for our study population was through the public mammography screening program. Thus, most patients presented with limited nodal metastatic disease and a relatively high proportion of micrometastatic nodal involvement. Moreover, patients with pre-planned ALND or neoadjuvant treatment were excluded.

Factors affecting the performance and false negative findings

Many previous studies and meta-analyses have addressed the sensitivity and specificity of AUS and/or ultrasonography-guided biopsy in the preoperative axillary evaluation. However, less is known about how various clinicopathological factors may affect the diagnostic utility of preoperative AUS.

Patients with axillary metastasis detected by AUS and biopsy have been reported to display poorly differentiated and large tumors[486]. Tumor size and LVI are recognized as the most important predictors of nodal metastasis and the likelihood of larger metastatic deposits often increases with tumor size. Accordingly, the sensitivity of AUS to detect metastasis increases with growing tumor size[409,487]. Thus, a normal AUS in patients with larger tumors and/or verified LVI should be cautiously interpreted[488,489].

Our data imply that histological grades affected the accuracy of AUS. In previous publications, a high tumor grade has been reported to be a predictor of nodal metastasis[263,270,275,329-332,490]. Recently, a prediction model was proposed to estimate the probability of nodal metastasis in patients with positive axillary ultrasonography, including a histological grade in addition to ultrasonographic features of the abnormal lymph node[491]. While other studies have reported no correlation between the tumor grade and accuracy of AUS[492], a study evaluating data from 1049 breast cancer patients concluded that patients with a false-negative AUS were more likely to harbor tumors of a high histological grade. The study advocated the cautious use of AUS to rule out nodal metastatic involvement in patients with high-grade tumors[493]. However, high tumor grade, although more aggressive, has been suggested to be associated with limited nodal metastatic burden but not high-burden disease[494]. It is further proposed that high-grade tumors mainly begin to hematologically disseminate during tumor growth and metastasize early, typically within the first 8 years following breast cancer diagnosis[495].

The current finding warrants investigations of the clinicopathological factors indicative of high FNR related to AUS. An FNR of 50% for AUS in the diagnosis of axillary metastases that arise from primary invasive lobular carcinoma has been reported[496]. This increased FNR for metastases arising from invasive lobular carcinoma has been confirmed by other publications[497,498]. These findings could be related to the histological behavior of the invasive lobular carcinoma metastatic deposits, which tend to be more dispersed throughout the axillary lymph node. In contrast, groups of metastatic cells observed in invasive ductal carcinoma are more unified[345]. Consequently, the architectural metastatic deformation of the axillary lymph node by lobular carcinoma deposits may not be as easily identified by ultrasonography.

Although ultrasonographic imaging with high frequency wavelengths could yield better resolution, it comes at the expense of diminished penetration and may reduce

accurate evaluation of the axillary lymph nodes[499]. Thus, one hypothesis suggests that obesity is accountable for a higher probability of false-negative results on AUS due to the thickened subcutaneous adipose layer in the axilla. The findings from Study I are in concordance with previous results showing that the BMI does not have a negative impact on the sensitivity of AUS[500-502]. While normal lymph nodes may be more difficult to distinguish in obese patients, metastatic hypoechogenic lymph nodes with thickened cortices can effortlessly be differentiated from the surrounding fat[502]. These findings suggest that AUS is a feasible diagnostic method, even in obese patients. The results are also supported by recently published data, which concluded that obesity itself is not an indication for additional axillary evaluation, if preoperative clinical examination of the axilla indicates a node-negative status[247].

Higher metastatic burden if preoperatively detected by axillary ultrasonography

While further studies are required to better assess how histopathological features of the primary tumor may alter the nodal architecture, it has been shown that the accuracy of AUS is highly correlated to the volume of the axillary disease. Compared with SLNB, the nodal metastatic burden identified by preoperative AUS-guided biopsy is reported to be greater, in both size of the metastatic deposit[503,504] and number of involved nodes[89]. This is logical because the sensitivity of AUS to distinguish nodal metastasis is lower than that of SLNB.

In a contemporary study cohort with >1000 breast cancer patients, FNRs for AUS were 46.2% for N1, 21.8% for N2, and 9.3% for N1 metastatic disease[493]. In accordance with our reported results, these data suggest that positive preoperative AUS and biopsy generally indicate N2 and N3 metastatic burden[500]. In addition, detecting one nodal metastasis by ultrasonography-guided biopsy has been reported to correlate with a mean of 5.2 metastatic nodes in the subsequent histopathological examination of axillary nodal dissection[505]. However, a recent publication revealed that patients with an abnormal lymph node at AUS but negative biopsy had a histopathological axillary nodal status similar to those displaying normal nodal sonographic features[506], with only 3.3% presenting three or more involved nodes.

Conflicting evidence on the clinical relevance after ACOSOG Z0011

AUS has traditionally been used to identify axillary nodal metastasis and to direct patients for ALND upfront without SLNB after metastatic findings. Early studies have demonstrated 1.4–45% omission of SLNB due to metastatic findings after AUS-guided biopsy[409,415,419,421,422,507-513]. However, the value of ultrasonography in the era of minimizing axillary surgery is indistinct, particularly in patients presenting with clinical T1-T2N0 disease, for which data from ACOSOG Z0011 indicated no recurrence or survival benefit with completion ALND, if SLNB revealed limited metastasis.

Findings from a study evaluating metastatic burden of T1-T2 tumors have suggested that 66% of patients with positive ultrasonography-guided biopsy have >2 positive axillary nodal metastases. The authors concluded that ultrasonography-guided biopsy can facilitate distinguishing patients ineligible for ACOSOG Z0011, and to direct these individuals to ALND upfront without SLNB[514]; other publications have confirmed these observations[515]. However, more recent findings have indicated that patients with an abnormal AUS but negative biopsy have an unaltered low rate of nodal metastatic involvement, and SLNB should be the surgical approach of choice[506]. A meta-analysis published in 2017 revealed that although patients with preoperative verified axillary metastasis by AUS and biopsy had significantly higher axillary burden, almost half of them had low-burden diseases and could be considered for omission of ALND according to the ACOSOG Z0011 criteria[516]. Thus, in patients with early breast cancer and a clinical node-negative status, proceeding to "fast track" ALND after positive AUS-guided biopsy has been criticized as overtreatment[517,518].

It is highly important to evaluate the ability of AUS to detect clinically relevant nodal metastasis. A recent study demonstrated a sensitivity of 76% for the detection of macrometastasis (>2.0 mm) by AUS alone in patients with clinical T1-T2N0 breast cancer. The administered adjuvant treatment in false negative cases after AUS has further been compared to the treatment recommendations with matched true negative cases. These treatment recommendations, derived from two medical oncologists blinded to the pathological lymph node status for cases with false negative AUS, were compared with those for matched true negative cases. This study reported that AUS did not influence the decision on systemic treatment, and suggested that information derived from surgical nodal staging is of decreasing importance for the choices of adjuvant treatment[488]. However, a Dutch multicenter study including >11000 patients demonstrated that patients <70 years old with clinically node-negative T1-T2 tumors and preoperatively verified nodal metastasis had worse survival than those with nodal metastasis verified by SLNB. These findings remained significant after adjustments for the number of involved lymph nodes and other established predictors of worse prognosis (e.g., tumor size and histological grade). All included patients underwent AUS as part of the routine work-up. The study concluded that a node-positive status after ultrasonography-guided biopsy is a significant prognostic indicator. It also suggested that the criteria of the Z0011 trial should not be applied to patients with positive ultrasonography-guided biopsy[486].

AUS has not been a prerequisite in the preoperative work-up preceding large randomized controlled trials of SLNB[123-125,127,130,519]. In the US, AUS has typically been performed in patients with gross palpable nodal disease[520]. In contrast, European guidelines[100,521] recommend that most breast cancer patients should undergo AUS as part of the routine diagnostic work-up, and that the patients should

proceed to undergo SLNB in case of a normal AUS or AUS-guided biopsy regardless of nodal palpability. Thus, it is crucial to recognize that the patient cohorts in studies on axillary management could considerably differ due to this bias in the preoperative work-up.

To summarize, the goal of AUS and ultrasonography-guided biopsy today is not only to verify the presence of nodal metastasis, but also to better quantify the metastatic load and improve the understanding of the prognostic value related to altered nodal features. The present debate questions the benefit of preoperative metastatic confirmation, since this may lead to unnecessary ALND. The goal is to better stratify patients to the required extent of axillary surgery and avoid axillary over- and undertreatment. Ongoing trials compare the omission of further nodal surgery in negative AUS with routine SLNB[26,226,227]. Furthermore, the feasibility of AUS after neoadjuvant treatment and its impact on sentinel lymph node surgery is another area of increasing interest[522,523]

Predicting the risk of nodal metastasis and the metastatic burden (Studies II-IV)

Evidence from landmark studies and ongoing trials regarding the role of axillary management is summarized in the Chapter "The required extent of axillary treatment". In this section, the aims to predict axillary nodal metastasis and the different extent of metastatic involvement are discussed, with special focus on clinical utility of the prediction models. Despite the accumulating evidence to endorse the de-escalation of axillary surgery, there are existing challenges to be considered. The Chapter "Clinicopathological characteristics and nodal metastasis" present each clinicopathological predictor included in the prediction models and the current evidence of their impact on the nodal status. In this section, general aspects and the main impact of key predictors are further discussed in relation to the results of Studies II-IV.

Aspects to be considered while deciding the extent of axillary surgery include the risk of symptomatic axillary metastases and that of underutilization of adjuvant treatments, because data on the axillary lymph node status have traditionally provided information to guide adjuvant treatment. Ultimately, the appropriate extent of axillary surgery should render regional control, and uncompromised disease-free and overall survival with minimized morbidity associated with the intervention. Therefore, to understand the clinical value of each prediction model and how these could complement the decision on the extent of axillary surgery, one must also recognize the morbidities related to adverse outcomes of axillary surgery.

Moreover, it is important to understand how adverse surgical outcomes, along with the fear of recurrence, may affect the patient's quality of life.

Adverse outcomes associated with axillary surgery

In the literature, SLNB is consistently reported to cause fewer complications than ALND[23,524-526]. Axillary surgery and lymphadenectomy can cause complications with sensory dysfunction, pain, and reduced mobility in the arm and shoulder[125,526]. A systemic review on shoulder and arm morbidity in 5448 sentinel node-negative breast cancer patients reported that a substantial number of breast cancer patients still experience shoulder/arm impairments more than 2 years after surgery[525]. The frequency of pain, numbness, loss of strength, and mobility ranged from 0 to 51% 2 years postoperatively[525]. Another systematic review reported that pain persisted in up to 51% of breast cancer survivors 8-10 years postdiagnosis[527].

Secondary lymphedema of the arm after breast cancer surgery and lymphadenectomy is lower after SLNB than after ALND[127,524]. However, the rates of lymphedema 2 years after SLNB have been reported to range from 0 to 27%[528-531] and increase over time[525], which adversely affects the quality of life[528,532].

After SLNB, the incidence of wound infection ranged between 0.8 and 10% during the first postoperative weeks[529,530,533,534], while that of hematoma ranged from 1.8 to 4.2% during the first week after the operation[529,530,534].

Quality of life aspects related to the extent of axillary surgery

A study with a long-term follow-up (12.5 years) reported that breast cancer survivors appear to have a quality of life similar to age-matched controls in most domains[535]. However, the risk of cancer recurrence is reported as a persistent stressor and affects the quality of life[536,537]. Considering the improved survival rates, the aspects of long-term morbidities related to the treatments and their impact on the quality of life are increasingly important.

A prospective cohort study investigated how ALND affected the quality of life in 990 breast cancer patients over 5 years. In this cohort, 38% of the patients reported arm problems 5 years after the surgery and the quality of life was significantly lower in patients with persistent morbidities[538]. The results on the effect on the quality of life comparing SLNB and ALND have been inconsistent. While some publications, including the ALMANAC trial showed a better quality of life for patients within the SLNB group[526,539], other publications did not report any effect on the overall quality of life with a short follow-up time[540,541]. These disparities could be explained by limitations in the standard questionnaires to entirely cover important viewpoints in this area.

Distinguishing between disease-free axilla and nodal metastasis

Prediction models with the aim to discriminate disease-free axilla from nodal metastatic disease (N0/N+) are presented in Studies II-IV. While all the models are internally validated, those in Study IV are also externally validated. The performance of the models, including clinicopathological variables, gene-expression (RNAseq) data, or a combination of these variables ranged from AUC 0.58 to 0.74 after validation across various breast cancer subgroups and prediction model techniques. Thus, the ability to classify nodal status was far from perfect and mirrors the complexity of factors related to axillary metastasis[542,543].

One of the first attempts to predict the axillary lymph node status by using prognostic indicators was reported by Ravdin et al. in 1994[284]. Although the addition of patient age, S phase, and PR as independent predictors of tumor size refined the estimation of nodal status, no patient subgroups could be distinguished with >95% chance of being nodal disease-free or harboring nodal metastasis[284]. It was concluded that these predictive models cannot alleviate the necessity of ALND for staging purposes as the nodal status influences adjuvant therapy choices. The shift toward SLNB reduced the complications of primary ALND. The focus of early axillary prediction tools was to identify the patients who were unlikely to benefit from ALND, as well as those with a high likelihood of metastatic disease in whom nodal metastasis would be missed by applying the more limited SLNB[262,264,266-268,328,544-547].

While improvements in imaging technologies for nodal staging are promising, the sensitivity and FNRs of these modalities alone for nodal staging remain insufficient to replace SLNB[548-550]. Because the information provided by nodal staging has been considered essential for guiding adjuvant therapy, SLNB is performed in all clinically node-negative patients, despite the observation that most of them have disease-free axilla[146,160]. For these patients, the invasive procedure has no therapeutic benefit. Most breast cancer patients present with low-risk tumors, node-negative disease, and excellent overall prognosis[380]. With growing insight into the importance of tumor biology[551] in the choices for adjuvant treatments, it has been suggested that surgical axillary status has a diminishing role[552]. Thus, there is an increasing interest to omit surgical staging in patients with a low risk of nodal involvement. If patients with a pathologically negative nodal status could be preoperatively predicted, the omission of nodal dissection would circumvent the adverse effects of axillary surgical staging and improve the quality of life.

Since the early studies more than two decades ago, risk models/nomograms to predict the nodal status have been developed in more contemporary populations[270,272,324,390,553]. However, conflicting evidence on the association of clinicopathological factors, gene expression data, and mode of detection of the extent of nodal lymphatic spread persists. Previous studies have reported diverse

performance of gene expression predictors to discriminate nodal metastasis, with AUCs between chance level to near perfect separation[554-559]. These discrepancies could result from differences in patient characteristics, cohort size, definitions of nodal disease, gene expression analysis platforms, and feature selection strategies.

The externally validated results from Study IV revealed that the addition of gene expression data to existing clinicopathological variables did not demonstrate clear superiority in predicting nodal metastasis in the evaluation groups defined by surrogate molecular subtypes. Furthermore, these results reinforced the central influence of tumor size and LVI as predictors of nodal metastasis, despite information on gene expression features.

Clinical utility - complementing risk estimation on omitting axillary surgery

Although the classification of the nodal status by the current models is of moderate accuracy, ROC curve analysis permits different cut-offs to be assigned, which are determined by the prevalent clinical setting[560]. To identify patients at a very low risk of nodal metastasis for selection to omit sentinel node biopsy, a cut-off could be set to achieve the highest possible negative predictive value in the prediction of N0. In Study III, the possible clinical utility of the N0/N+ prediction model in reducing unnecessary SLNB was assessed. By adjusting the cut-off value according to a maximum NPV or an FNR of 5–10%, the corresponding SLNB reduction rate would be 8–27%.

Several randomized trials, e.g., SOUND[26], INSEMA[226], and BOOG 2013-08[227], are now addressing the possibility of omitting surgical axillary nodal staging. However, the safety of the inclusion criteria in these trials is unconfirmed. Therefore, predictive models for N0/N+ could complement the identification of the patients who are more likely to have a disease-free axilla. Moreover, the prediction of the nodal status could minimize the risk of underutilization of neoadjuvant and/or adjuvant therapy.

Predicting limited disease

There is accumulating evidence that ALND can be safely omitted after limited disease in the sentinel node(s)[561]. In particular, the results of the ACOSOG Z0011 trial[23,27,562], which favor less extensive axillary surgery, have influenced surgical axillary management[563]. These results highlight the importance of distinguishing low- from high-burden metastatic diseases.

The results from Studies II and III demonstrated that predicting limited disease was more challenging than predicting disease-free axilla or high-burden disease. However, identifying the presence of limited metastatic burden is valuable. On an average, 2–3 lymph nodes are removed if SLNB alone is performed for nodal

staging,[124,564] and most metastatic nodes are identified with the excision of the first three sentinel nodes[143]. Thus, an accurate prediction of ≤3 metastatic lymph nodes could spare most node-positive patients from completion ALND. In Study II, the end-points of the analyses were selected after applying the criteria from the ACOSOG Z0011 trial, with the aim to predict 1–2 nodal metastasis in patients with T1-T2 breast cancer; a bias-corrected AUC of 0.69 was obtained. In Study III, an ANN-based model was developed, with the aim of predicting 1–3 metastatic lymph nodes in patients with clinically node-negative axilla; this model yielded an internally validated AUC of 0.71.

The results of the ACOSOG Z0011 trial have been practice-changing[217-219], and the outcomes have been integrated into clinical guidelines[220]. However, severe criticism of this trial has been voiced, which includes the study design, patient inclusion, and radiotherapy approaches[166]. In the different trials addressing the possibilities of a limited surgical approach[23-25,133], there are also important differences in the histological techniques for examining excised lymph nodes, which could affect the conclusions and outcomes[565]. One of the main weaknesses of the randomized trials aimed to address the limitation of axillary surgery is that they were conducted without considering distinct tumor biology characteristics (e.g., molecular subtype and markers for proliferation, such as grade or Ki-67).

Clinical utility - complementing risk estimation on omitting ALND

The prediction models in Studies II and III aimed to present the pooled impact of breast cancer biology, mode of detection, and clinicopathological data on limited nodal metastatic involvement in breast cancer patients who underwent breast-conserving therapy or mastectomy. At present, the necessity for completion ALND in patients with a low-burden metastasis remains a major dispute, although the safety of omitting completion ALND in selected patients is demonstrated by the aforementioned randomized trials (Chapter "The required extent of axillary treatment"). Again, for those who do not meet the inclusion criteria of these trials, and perhaps also for some who do, completion ALND may be of a therapeutic value. The significance of locoregional control cannot be overlooked, particularly since it has been proven that other aspects of improved regional control (e.g., radiotherapy after breast-conserving surgery) could result in a decrease in breast cancer-related mortality[108]. The EBCTCG publication in 2014 with an updated meta-analysis on the effects of postmastectomy radiotherapy provided further evidence for the effectiveness of locoregional control for reducing the risk of recurrence and mortality in patients with 1–3 positive nodes[566]. However, in 2016, the updated American Society of Clinical Oncology (ASCO) guidelines acknowledged the controversy regarding the value of locoregional therapy for patients with T1-T2 disease and limited nodal disease (1–3 positive nodes)[567]. It was stated that patient characteristics and biological characteristics of the tumor should be considered

when selecting the extent of radiotherapy. Although the ASCO panel recognized that risk-adaptive models could guide clinicians in the choices on the amount of necessary treatment, no specific model was endorsed.

Thus, for patients with low-burden metastatic disease in the axilla, multiple treatment strategies have been suggested by different trials (e.g., SLNB alone, completion ALND, and/or regional radiotherapy). The importance of the pooled data on patient-related factors and tumor-related features has been increasingly endorsed for guidance on the extent of axillary surgery and adjuvant treatment. If validated, prediction models for limited disease could complement the risk estimation in sentinel node-positive patients and better guide axillary management. Mature follow-up data on the conducted randomized trials and outcomes of ongoing studies addressing the risk and benefit of limited axillary therapy are awaited.

Predicting heavy-burden disease

Prediction models to discriminate the involvement of ≥3 and ≥4 axillary lymph nodes are presented in Studies II and III, respectively. Although the interest in recognizing heavy-burden axillary metastasis has been long-standing, focus on the use of these models to discriminate high-volume disease has been altered over the last decade.

Since the shift toward SLNB as primary routine nodal staging, it was confirmed that for most patients with early-stage breast cancer with positive sentinel node(s), no further metastatic nodes were identified on completion ALND[568]. Before the publication of the ACOSOG Z0011 trial results, axillary risk models aimed to estimate non-sentinel node involvement. Van Zee et al. suggested a risk predicting model/nomogram that would produce an estimation of the risk of non-sentinel node metastasis[381]. This Memorial Sloan-Kettering Cancer Centre (MSKCC) nomogram has since been evaluated[388,569-571] and numerous other models predicting non-sentinel node involvement have been developed[271,382,384,385,572]. Most prediction models include features of the sentinel node metastasis (e.g., dimensions of the metastasis and extranodal extension) in addition to commonly included estimates of tumor size and LVI. However, retrospective studies involving patients with positive sentinel nodes who underwent completion ALND have implied that the performance of these monograms is insufficient for clinical practice[570,573].

The current prediction models include variables that are feasible to obtain in the preoperative setting and are not dependent on the characteristics of the sentinel nodes as a benchmark. Internally validated performance of the models ranged from AUC 0.75 to 0.79. These results implied that predicting a high-burden disease is easier than discriminating low-burden metastatic involvement or identifying disease-free axilla. This hypothesis is further supported by comparing the

components of the ANN-based models for the three different nodal end-points. While ANN-based models for N0 and N1 constitute a complex integration of predictors as input variables, only six input variables are predictive in the ANN structure for heavy-burden disease (N2): tumor size, LVI, ER status, histological type, and multifocality.

With the publication of the ACOSOG Z0011 trial results, the clinical value of nomograms predicting non-sentinel node metastasis or the total axillary metastatic volume has been questioned. The outcome of the trial may propose the sparing of ALND regardless of the predicted probability of a heavy-burden disease. However, this trial enrolled a highly selected population of patients and determining Z0011 eligibility is not achievable in a preoperative setting, since it is dependent of the sentinel node status.

Clinical utility - decisions on neoadjuvant therapy, ALND, and adjuvant regimens

Predicting the risk of heavy-burden disease by accounting variables obtainable in a preoperative setting could identify patients who would benefit from neoadjuvant therapy and those for whom ALND would be of a therapeutic value and not merely helpful in staging. The meta-analyses of long-term outcomes among 100,000 women comparing different polychemotherapy regimens concluded that neoadjuvant treatment should only be provided if the patient has an indication for adjuvant therapy[176]. In this context, an early estimation of locoregional tumor load is important. Prediction models that weigh the significance of each variable outperform clinicians in the estimation of heavy metastatic spread in the axillary basin[574,575]; thus, these models could complement treatment decisions after triple-diagnosis.

In the ACOSOG Z0011 trial, patients who underwent mastectomy were excluded. Differentiating the metastatic involvement of ≥4 lymph nodes is essential according to the ASCO guidelines for planning adjuvant postmastectomy radiotherapy[567]. Similarly, the Swedish guidelines for treatment suggest that a wider irradiation field (partial inclusion of the parasternal internal mammary nodal regions) could be considered for patients who have undergone mastectomy and display >3 metastatic axillary lymph nodes. Furthermore, the preoperative prediction of high-volume tumor burden to determine the probability of adjuvant radiotherapy is also important in guiding choices on immediate reconstruction after mastectomy due to the effects of irradiation on outcomes of breast reconstruction[576].

In summary, preoperative prediction of heavy-burden disease could guide treatment options, including the need for neoadjuvant treatment, axillary dissection, and the extent of required locoregional radiation.

Predictors of nodal status: anatomy, biology, and non-linear associations matter

Studies II–IV reinforced tumor size[146,270,272,577] and LVI[266,272] as the two variables most strongly associated with nodal status. These variables were central in the prediction of any nodal status output; disease-free axilla, limited axillary metastasis, or heavy-burden disease, regardless of model selection. This is in accordance with previous meta-analysis on predictive factors for additional nodal metastasis in the axillary basin after a positive sentinel node[578]. Historically, the assessments of breast cancer prognosis and choices of surgical and adjuvant treatments have been driven by anatomy, defined by the size of the primary breast tumor and magnitude of the disease.

Large studies investigating the association between tumor size and lymph node status before the SLNB era reported the frequency of lymph node metastasis as 10–29% for T1 cancer[262,544,579-583], 39–59% for T2[262,580,583], and 71–80% in T3[262,580]. These findings might suggest that axillary lymph node metastasis is merely a reflection of the chronological age of the breast tumor, which is assumed to be displayed through its size[584].

The less-than-perfect association between size of the breast tumor and its ability to metastasize to the axillary lymph nodes was demonstrated in Study II. Here, the increase in tumor size was less often associated with metastatic nodal involvement in the TNBC subtype than in the non-TNBC subtypes. Similarly, the publication by Hernandez-Aya et al. provided evidence for a non-linear association between tumor size and the risk of nodal involvement for TNBC tumors, showing that once axillary lymph node metastasis is established, patient outcomes were not influenced by the quantity of metastatic nodes[585]. These results were also in accordance with previous findings, which suggested that the survival outcomes among patients with TNBC tumors are not linearly correlated with the tumor size or nodal status[586,587]. Similarly, Wo et al. proved that in heavy-burden axillary lymph node metastatic disease (N2), patients with T1a tumors (>1 mm but ≤5 mm) had higher breast cancer-specific mortality than those with T1b tumors (>5 mm but ≤10 mm). However, it was observed that after a certain threshold, breast cancer-specific mortality increases with increasing tumor size[588]. Notably, particularly among patients presenting with ER-negative tumors and N2 nodal burden, those with T1b cancer experienced significantly lower breast cancer-specific mortality than did those with the smaller T1a tumors. These findings reinforced the importance of tumor biology related to axillary metastatic potential.

The association between breast cancer subtypes, as defined by the surrogate immunohistochemical criteria of the St. Gallen consensus, and axillary status were presented in Study II. Most patients with TNBC tumors had disease-free axilla, and the TNBC subtype was more likely to be node-negative than the Luminal A-like

subtype. This was supported by Study III, showing that positive ER and PR statuses were predictive of nodal metastasis. Moreover, a non-linear association between the ER status and volume of axillary metastatic involvement was observed. In Study IV, higher proportions of the luminal B intrinsic features were observed in predicted node-positive ER+HER2- and HER2+ tumors, while upregulated basal-like features were observed in node-negative TNBC tumors; this is in accordance with previously reported results on tumors of basal-like subtype[587]. Machine-learning approaches in Study IV further identified five genes to be ranked higher than LVI and tumor size as the most dominant predictors of nodal metastasis in TNBC tumors. Altogether, these findings verify the significance of biological markers and molecular subtypes, in addition to the chronological tumor age and size, for the acquisition of nodal metastatic potential. However, transcriptional heterogeneity across molecular breast cancer subtypes for N0/N+ was displayed in Study IV, which at the same time reflect the challenges for expression-based predictors to accurately discriminate the nodal status.

Current and previous findings indicate that the axillary metastatic ability is, to some extent, predictable (highly associated with tumor size and LVI), and unpredictable at the same time (modified by biological markers). Correspondingly, ANN-based models to predict the extent of nodal metastatic burden in Study III emphasize nonlinear associations between preoperatively obtainable clinicopathological variables and axillary metastasis. Tumor size, LVI, and multifocality displayed approximately linear correlation patterns relative to the risk of any nodal metastasis, low-burden and high-burden disease. However, other predictors that were found of significance for the prediction of the nodal status and metastatic volume (age, histological type, ER status, Ki-67 values, mode of detection, and tumor localization in the breast) revealed various degrees of dynamic associations to each nodal status end-point. The superior discriminatory performance presented by the ANN algorithms compared with the matching multivariable logistic regression models for each nodal status output highlights the importance of recognizing the non-linear molecular mechanisms underlying the complex biology of axillary metastasis.

Models of metastatic spread often describe an intricate interaction between seed and soil elements, which include various aspects of tumor and microenvironment factors[589], one of which is tumor proliferation. Proliferation-related prognostic transcriptional programs[238,590-593] are particularly recognized in luminal breast cancer. In Study IV, a higher expression of proliferation-related genes was displayed for ER+HER2- and HER2+ subgroups, with a predicted node-positive status. Moreover, enrichment of proliferation-associated gene ontology terms was observed in the prediction models based on gene expression data. These findings are in accordance with those of Study III, which showed the significance of Ki-67, a measure of the proliferative activity of breast cancer tumors, in predicting any nodal metastasis and limited nodal metastatic burden.

Although the nodal metastatic features recognized by the predictors for ER+HER2- subgroups increased prognostic impact over the pathological nodal status alone, it is important to critically evaluate these results relative to traditional variables of proliferation. Our findings suggest that the prognostic impact obtained from the proliferative features of the predictors is related to the prognostic value of histological grading. For patients with limited nodal metastatic burden (1–3 metastatic lymph nodes), increasing evidence supports the prognostic value of multigene assays. Meta-analyses of microarray-based expression profiling studies have proved that the prognostic impact of multigene assays primarily stems from proliferation-related genes[591,594]. The randomized prospective MINDACT trial, which evaluated the utility of the 70-gene signature (MammaPrint), suggested that the biological characteristics of the breast tumor are significant with respect to choices of adjuvant therapies and outcomes even among those with limited nodal metastatic burden. Recently, the 21-gene recurrence score (Oncotype DX) was revealed to significantly predict the risk of locoregional recurrence in patients with node-positive, ER-positive breast tumors after adjuvant chemotherapy and tamoxifen. These findings could further guide the choices of comprehensive locoregional radiotherapy. Hence, evidence is accumulating in favor of applying gene expression profiles for decisions on adjuvant treatment[184,595,596]. However, there are no direct comparisons of established molecular signatures in the estimation of axillary metastatic burden to date. In the overall validation cohort in Study IV, the predictor that combines gene-expression features and clinicopathological characteristics demonstrated the highest accuracy in predicting nodal metastasis, attaining AUC of 0.72. Thus, the ability to classify the nodal status was suboptimal. The addition of gene expression data to existing clinicopathological variables did not demonstrate a clear superiority in predicting the nodal status. However, to fully validate the potential of transcriptional profiling for nodal prediction, a more precise stratification of the breast cancer subgroups could be relevant. Moreover, further investigations with focus on the subset of patients with early extensive lymph node metastasis may help in elucidating the genetic or molecular differences that contribute to acquisition of an early metastatic potential.

Conclusions

- Accuracy of axillary ultrasonography is highly dependent on the size of the nodal metastatic deposit and the number of involved nodes. Using axillary ultrasonography to detect low-burden metastatic disease warrants caution.
- Patients with preoperatively verified nodal metastasis by axillary ultrasonography-guided biopsy harbor N2 disease more often than those having normal axillary ultrasonography but presenting with metastatic sentinel lymph nodes.
- Estimation of axillary nodal burden using preoperatively obtainable predictors is achievable. Patients least likely to have nodal metastasis might be safely spared surgical axillary staging using the maximized negative predictive value as cut-off to delineate disease-free axilla.
- Artificial network models show promise in distinguishing disease-free axilla, limited axillary metastasis, or heavy-burden disease. Nonlinear association between the clinicopathological variables and axillary metastatic involvement should be taken into consideration.
- Patients with T1-T2 triple-negative breast cancer (TNBC) are more likely to present with node-negative disease compared to those diagnosed with Luminal A-like breast tumors.
- The increase in tumor size is less often associated with metastatic nodal involvement in the TNBC subtype than in the non-TNBC subtypes.
- The addition of gene expression data to routine clinicopathological variables did not demonstrate a clear superiority in predicting nodal metastasis across the surrogate molecular subtypes based on the ER, PR, and HER2 status.
- Higher proportions of the Luminal B intrinsic features and proliferation-related genes were observed in ER+HER2- and HER2+ tumors with predicted node-positive disease using clinicopathological and gene expression predictors (alone or in combination). Low expression of basal-like markers was detected for TNBC tumors with predicted nodal metastasis.

“Student: Dr. Einstein, Aren't these the same questions as last year's final exam?
Dr. Einstein: Yes; But this year the answers are different.”

Future perspectives

Nodal metastatic process - reevaluate the "bystanders"

There is evidence of differences in biological patterns of lymphatic versus hematogenous metastatic spread[597] which reflects the complex interplay between cancer cells and their microenvironments. Significant advances have recently been made in understanding lymphatic biology, lymph node microenvironments, and immunology in the process of lymphatic metastasis[598]. Lymphatics, and especially lymphatic endothelial cells, should no longer be thought of as passive bystanders in nodal metastasis, but rather active players in the intracellular and intercellular processes of metastatic development through the production of cytokines and chemokines[599-601]. The induction of lymphangiogenesis and formation of a premetastatic niche in the sentinel lymph nodes is still poorly understood[602]. Researchers are currently investigating ways to target lymphatic niche factors that may promote lymphatic spread, and are evaluating the utility of anti-lymphangiogenic therapies in preventing recurrence (e.g., targeting VEGFR-3 and photodynamic therapy to eradicate in-transit lymphatic metastasis)[603]. This, together with a better understanding of tumor dormancy and control of metastatic outgrowth, will help optimize a more personalized approach for axillary management in patients.

Axillary imaging in the era of surgical de-escalation

Much remains to be learned on how various clinicopathological factors could affect the diagnostic utility of preoperative axillary ultrasonography (AUS). While AUS is increasingly implemented as part of the routine diagnostic work-up, further studies are needed to better assess the factors that impact the reliability of this imaging technique. The absence of conclusive guidelines in categorizing and reporting AUS findings is complicating the interpretation and comparison of AUS findings. Therefore, improved quantification of abnormal axillary nodes on AUS, and standardized description and classification of the aberrant nodal morphology are needed.

Currently, prospective studies are ongoing to show the value, as well as limitations, of AUS and AUS-guided biopsy for axillary management. It would be interesting to outline how patients should be directed after preoperative verified node-positive axilla, and further translate these findings into therapy guidance: neoadjuvant therapy, SLNB or "fast track" ALND.

The high FNR of AUS and AUS-guided biopsy signify technical limitations of preoperative imaging and sampling to accurately diagnose low-burden disease. AUS-guided core-needle biopsy has been suggested to be more sensitive than fine-needle aspiration biopsy[604], and there is room for new technologies to improve the accuracy of preoperative nodal evaluation.

Newer techniques, e.g., ultrasound elastography, have proven promise in assessing the extent of axillary metastatic disease burden and discriminate between benign reactive nodes and metastatic involved nodes. The higher "stiffness" of a malignant involved lymph node and a change in elasticity can be detected using elastography, a function often accessible on modern ultrasound systems[605]. Additionally, the contrast-enhanced ultrasound (CEUS) technique has been shown to improve ultrasonography-guided biopsy of sentinel lymph node. A microbubble suspension contrast agent is intradermally injected into the periareolar area, enters the lymphatics, and can be traced to the sentinel lymph nodes[606]. A few studies have addressed the performance of a combination of FDG PET/CT together with US or MRI, to facilitate identification and excision of suspicious axillary lymph nodes[607]. Furthermore, a computational approach to categorize axillary nodal metastasis in ultrasonographical images has been described[608]. Nevertheless, for clinical benefit, results from these emerging technologies need to be validated. Another issue to address is how to improve marking or guide clipping of the lymph nodes that will enable a more targeted axillary management after neoadjuvant treatment.

Prediction of nodal status - the quest for the Good, the Bad, and the Ugly

The present findings on predictors of nodal metastatic involvement are worth pursuing, and the next significant step is to validate current results. Prediction models in Study II are provided in a user friendly graphical format, which readily allows clinicians and researchers to externally validate the nomogram performance. In Study III, possible cut-offs in the prediction model for disease-free axilla suggest the utility of the model in reducing unnecessary SLNB. However, the ability to predict disease-free axilla needs to be validated externally and prospectively. The ANN-algorithm should thus be adapted into a graphical interface and be made publicly available to facilitate this validation process. Even though the addition of

gene expression data to existing clinicopathological variables in Study IV did not show a clear advantage in predicting nodal status, transcriptional profiling for nodal status prediction should be addressed further. Little is known about the association between microRNA expression or proteomic profiling, and axillary lymph node status. While evidence is increasingly available for the application of gene expression profiles for adjuvant guidance, there are no studies yet addressing the performance of the established multigene assays in the estimation of axillary metastatic burden. Therefore, it would be interesting to assess the discriminatory abilities of these prognostic signatures in the prediction of nodal status, and evaluate the results in the context of findings from Study IV. Another appealing avenue for future prediction models on axillary management would be to include the pooled effect of Radiomics, clinicopathological risk predictors, and proliferation markers.

Accurate preoperative estimation of breast cancer characteristics is essential in providing precise variables for prediction models addressing axillary management. Thus, it is of interest to further investigate the concordance between preoperative (e.g., by core-needle biopsy) and postoperative histopathological characteristics.

Forthcoming mature data from randomized trials will impact the definition of "good" or "worrisome" nodal status in the clinical setting. The keystone of a successful clinical predictive model is the ability to adjust its estimates to current knowledge. The possibility of limiting axillary surgery based on quantifying of involved lymph nodes has attracted significant attention in recent years. However, future models on axillary management may not be just weight predictors for merely quantitatively predicting metastatic burden, but may also integrate other essential measurements such as the risk of adverse surgical outcome and quality of life end-points.

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Today, the majority of patients newly diagnosed with breast cancer present with low-risk tumors and excellent overall prognosis. With earlier detection, the overall node-positive rate in primary breast cancer has dropped to 15-30%. Thus, for most patients with breast cancer, the routine surgical nodal staging by sentinel lymph node biopsy has no therapeutic benefit. To improve treatment for those with breast cancer, a better understanding of tumor biology related to metastasis is urgently needed. This knowledge, along with the incorporation of axillary imaging technologies and prediction tools, could facilitate a more tailored approach to axillary management.

This thesis presents novel nomograms to estimate disease-free axilla, limited axillary nodal metastasis and heavy-burden metastatic disease alongside prediction models based on machine learning techniques, including those from artificial neural networks. Finally, the clinical utility of the prediction tools to estimate nodal metastatic burden is discussed in the context of current evidence on axillary treatment.

Looket Dihge M.D.

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences Lund

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