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Lymph node infiltration, parallel metastasis and treatment success in breast cancer

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ABSTRACT

Background: The number of axillary positive lymph nodes (pLN) is the most important clinical prognostic factor in breast cancer (BC). To date, there is limited knowledge of LN-spreading and metastasization (MET).
Patients and methods: In the Munich Cancer Registry, 30,170 hormone receptor positive BC patients were analysed for the variation in tumor diameter (TD) and number of pLNs. A total of 144 combinations were described with Gompertz functions for each LN-subgroup and linked with patient outcomes, MET and 20-years survival.
Results: Every additional millimeter of BC diameter decreased the likelihood of 0pLN-status by 1.6% −0.3%. The infiltration accelerates from the 1pLN and the percentage of successive pLNs subgroups becomes smaller. BCs with increasing TDs continuously reduce the proportion of 0pLN-status and increase it with >10pLNs. The proportion of 1−10 pLNs at 10 mm is 16% and increases to 50% with prognostically favorable 1-2pLNs of 75% and 40%, respectively. After 20 years, tumor-specific mortality is about 17% for 0pLNs, twice that for 1pLN, and 3 times higher for 4−5 pLNs. The more LNs are positive, the less survival is affected. The subgroups with 0/1pLNs cause 41/16% of all cancer related death.
Conclusion: The number of pLNs is an epiphenomenon of the onset and chronometer for the duration of TCs disseminating from growing BCs. METs are initiated parallel to LNs by PTs and not caused by pLNs. This LN process without cascade-like MET initiation should be generalizable to all common solid tumors.

1. Introduction

Like any malignant primary tumor (PT), breast cancer (BC) can develop metastatic competence due to genomic changes during growth. Disseminated tumor cells (TCs) initiate local, regional and distant MET. The most important clinical prognostic factor is the occurrence of regional dissemination, indicated by the number of axillary positive lymph nodes (pLNs). However, our current knowledge of the spatiotemporal evolution of LN invasion and MET is limited [1]. With our data we would like to contribute to the LN-topic: Can the sequential spreading of TCs into the LNs caused by growing BCs be described in detail, and what can be inferred about cause and time of the crucial evolutionary steps of MET?

2. Materials and methods

In the Munich Cancer Registry [2], which has been listed in "Cancer Incidence in five Continents" [3] since 1998, reliable pathological data on tumor diameter (TD) and number of pLNs as well as long-term follow-up for more than 20 years are available from a population of 4.8 million. Exclusion criteria for the current data analysis were in situ BCs, primary advanced BCs, neoadjuvant treatments, hormone receptor (HR) negative BCs and earlier or synchronous second primary malignancies. The resulting data set is a molecularly homogenous subgroup of HR positive BCs that represent about 55% of all BCs today.

Data from 3 decades is summarized, contributing to an over-representation of large BCs which are treated neoadjuvantly today.

Abbreviations: Gf, Gompertz function; HR, hormone receptor; (p)LN, (positive) lymph node; MET, metastasis; PT, primary Tumor (breast cancer); TD, tumor diameter.

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Follow-up is available by incorporating all death certificates from the region. In the case of cancer-related death, 75% of MET are annotated during the course of disease. Despite missing values, it is robust data for the analysis of the LN-dependent MET process as has already been shown [4,5].

For LN-infiltration there are 144 frequencies from 30,170 patients for the two combined items: number of pLNs with 12 subgroups (0–10, >10pLNs) and TD with twelve 5 or 10 mm intervals. The relationship is basically S-shaped between 0 at small TD and an upper limit at large TD and can be described with Gompertz functions (Gf). The Gf is a sigmoid asymmetric function and has 3 regression coefficients: a: an asymptote that is approached gradually for larger BCs, b: the shift on the TD axis, and c: the growth rate. Percentages of each pLN depending on TD are successively cumulated and a Gf is fitted: For each LN-subgroup and each TD the percent of BCs having npLNs and more is provided ($Gf_{\geq npLN}$ (%) = a*exp(-b*exp(-c*TD)). For better presentation, the complement to 100% is shown and results e.g. for 1pLN: $y_{\geq 1pLN}(\%) = 100\text{-}Gf_{\geq 1pLN}$: (a:67.8,b:3.5, c:0.051,TD)) (Fig. 1a-b).

The relationship between LN-infiltration and MET is shown with Kaplan-Meier curves for the relative survival from diagnosis, for survival after MET and with distributions of the MET-free survival time. The impact of independent prognostic factors on overall survival is investigated by Cox proportional hazards models. Statistical analyses were performed by using SAS V 9.4 and R V 3.1.3.

3. Results

The distribution of pLNs as a function of TD is shown in Fig. 1a. As the pLNs increases, the prevalence of the subgroups decrease, that is, the infiltration of the LNs is accelerating. In Fig. 1b, the observed and fitted cumulative frequencies of the pLNs are arranged. All regression coefficients result in significant adjustments ($p < 0.001$).

LN-infiltration is a dynamic process that becomes quantitatively accessible with the Gompertz regressions. For small BCs, one out of 100 patients becomes LN positive with every additional millimeter of TD. At 15 mm, a maximum of 1.8 patients will have a tumor that infiltrates LN for the first time, dropping to 1 at 36 mm and 0.5 at 50 mm TD. 25% of patients remain LN negative even with large PTs ($y_{\geq 0pLN}(\%) = 100 - Gf_{\geq 0pLN}$ (a: 75.4, b:2.82, c: 0.063, TD)) (Fig. 1b). If another LN is infiltrated, an influx into the subgroup with npLNs from the (n-1)pLN subgroup or an outflow to the (n+1)pLN subgroup occurs. Despite this transfer dynamic, the proportion of each subgroup remains stable. The continuous infiltration can also be described by the sum of all pLNs dependent on the TD ($y_{npLNs} = Gf$(a: 646, b:3.91, c:0.041, TD)). In 100 patients, initially 2 LN per mm TD are additionally infiltrated, this increases to 8–10 LN per mm with TD between 20 and 45 mm. At 66 mm TD, there are about 500 pLNs in 72 out of 100 patients with pLNs.

In Fig. 1b, tumor-specific fifteen-year survival shows a dependency on TD that conforms with the 0pLN-subgroup. A fictitious regression function $y_{\geq 0^*pLN}(\%)$ = (100-$Gf_{\geq 0^*pLN}$: (a:85.8, b:2.63, c:0.078, TD)) separates another status denoted 0pLN*, whose proportion is just as large as that of 1pLN and is intended to describe the proportion of already initiated MET in the 0pLN-status.

The same distance is suggested by tumor-specific survival with 0 and 1pLN. Fig. 2a shows that after 20 years in the 0pLN-status, just as many METs are initiated as with 1pLN due to the further growth of the PT, i.e. without excess mortality from the first pLN. Both groups cause 41/16% of all deaths. As the number of pLNs continues to increase, the increase in MET or tumor-related mortality becomes smaller and smaller. In contrast, survival from MET (Fig. 2b) is only slightly correlated with the number of pLNs. We have no plausible explanation for the approximate 9 months shorter median survival in >7pLNs, especially since the 5-year survival varies between 10% and 30% and the ranking corresponds exactly to the number of pLNs.

A stronger correlation exists with the MET-free survival time (Fig. 2c). Only historical data with 20 and more years of follow-up can show that at least 20% of the METs from 0pLN-BCs occur after 10 years or 50% up to 5 years. For more than 10pLNs, these frequencies are 5% after 10 years or 50% in the first 2.9 years. Primarily advanced BCs are excluded in Fig. 2c. The M1 fraction increases with the number of pLNs (Fig. 2d) and is also distributed in an S-shaped curve. Table 1 summarizes the results of Cox modeling for overall survival. The hazard rates for the number of pLNs show the same observed survival at 11–19 pLNs as well as the striking

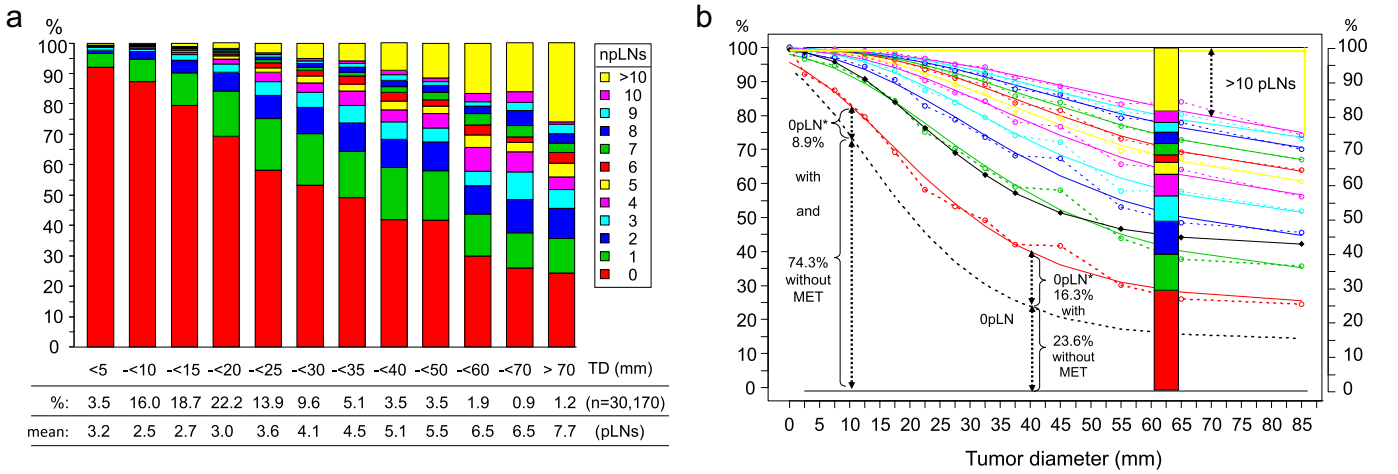

Fig. 1. a: Distribution of positive LNs, frequencies (%) and mean number of pLNs in dependence on TD for HR positive BCs (see cohort definition) (n = 30,170). 1 b: Proportion of n and more positive LNs in dependence on TD. Cumulative observed data (dotted lines) and fitted Gompertz functions (Gf) (solid lines) show for each TD the percentages with ≥npLNs. The stacked bar is from Fig. 1a. The black line is the fitted Gf for the observed tumor specific 15-year mortality (diamonds) depending on TD. (MCR data since 1988: n = 36,686, $y_{15yS}(\%) = 100\text{-}Gf_{15yM}$(a: 58.4, b: 4.46, c:0.071,TD)). The dotted black line estimates the proportion of patients (0pLN*) with METs initiated during 0pLN-status by MET competent tumor cells of PTs. (100-Gf0pLN*(a:85.84, b:2.63, c:0.078) The length corresponds to the MET risk of 0pLNs and 1pLN in Fig. 2a. (The various Gfs can be easily plotted with the free programming language R: x < - seq(0,90,1); y < – 100-85.8*exp(-2.6*exp(-0.078*x)); plot(x,y,ylim = c(0,100)); lines(x,100-67.9*exp(-3.5*(exp(-0.051*x))),col = 2); lines(x,100-75.4*exp(-2.8*(exp(-0.063*x))),col = 3)).

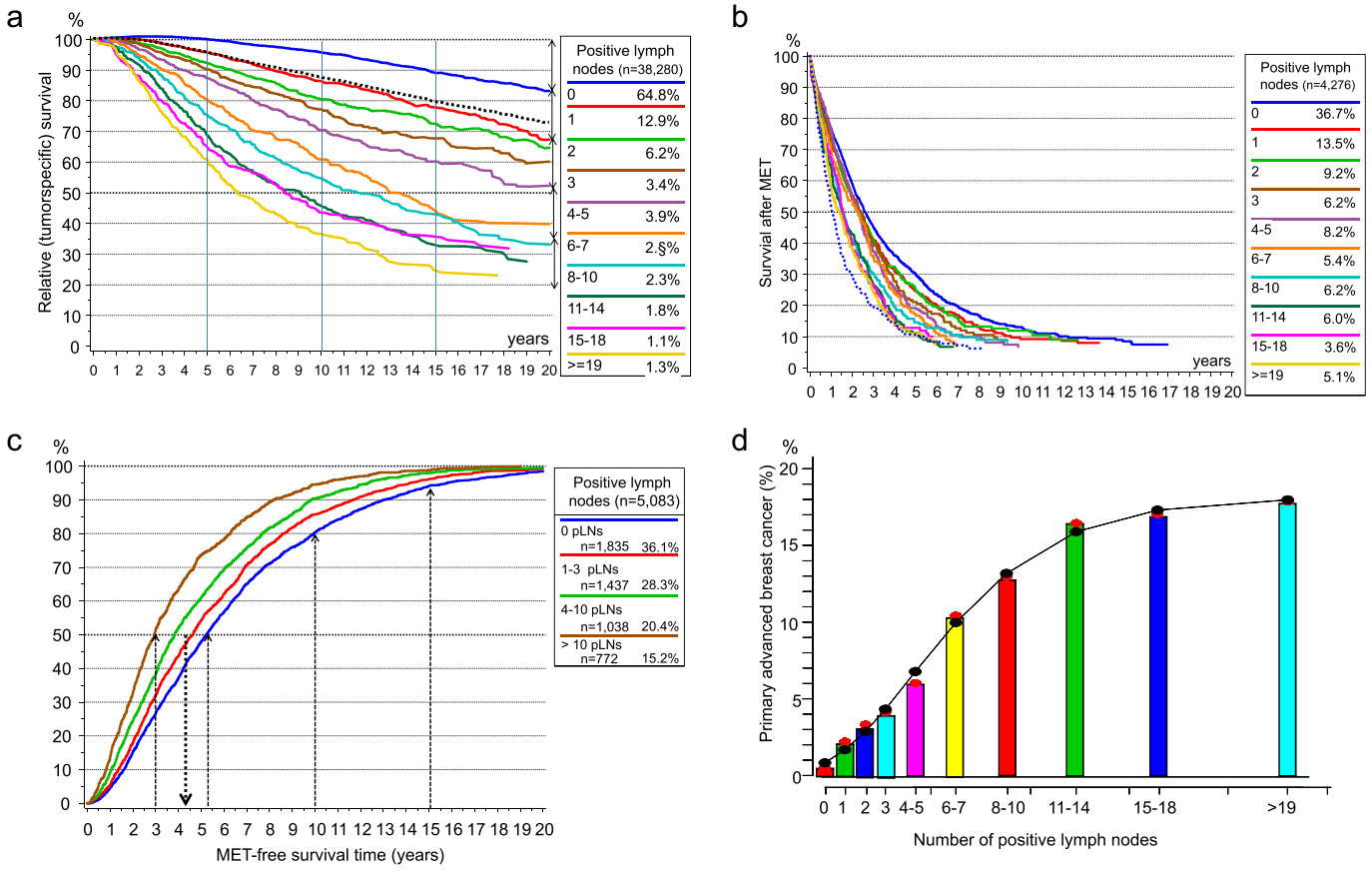

Fig. 2. **a**: Kaplan–Meier estimates for 20-year relative survival[#] depending on the number of pLNs (n = 38,280, primary M0, HR positive, since 1988, the dotted black curve shows the survival of all these patients) Same-sized arrows show that increasing numbers of pLNs have a diminishing mortality risk (15-year-relative survival (%) = 81.7 * exp (−0.0802 * n) (n: number of pLNs $0 \leq n \leq 25$)). These are historical data. Since 1998, population-based 15-year survival has already improved by more than 5% in all subgroups. Survival-curves are drawn up to 10 patients under risk. The 5 arrows illustrate the relationship between mortality increase to 80% and the number of pLNs. [#] The relative survival is an estimate for tumor-specific survival and is calculated by dividing the overall survival after diagnosis by the survival observed in the general population with comparable age distribution. **2b**: Overall survival after MET depending on the number of pLNs (n = 4276) and after MET from HR negative BCs with 0pLNs (dotted curve, n = 309). **2c**: Distribution of MET-free survival time in dependence on the number of pLNs. The dotted line marks the weighted median at 4.4 years, dashed lines prominent points, e.g. 20% of MET have a MET-free survival >10 years. **2d**: Primary advanced BCs in dependence on the number of pLNs (n M0:38,280, n M1:1059). The metric distances of the bars show the sigmoid curve with lower MET risk for increasing pLNs. (dotted line: observed data, red line: fitted Gompertz-function $y_{M1}(\%) = Gf_{M1}$ (a = 18.2, b = 2.99, c = 0.25, npLNs: 0–25). Again, these are historical data because today primarily advanced BCs are usually treated systemically first and therefore number of pLNs are missing. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

difference between 4-5 and 6–8 pLNs in the defined subgroup. But pLNs do not explain the prognosis alone. The hazard rates for the pT-categories increase and represent METs, which cannot be explained by pLNs.

4. Discussion

To begin with, our analyses provide evidence that LN-infiltration can be modeled with detailed data on pLNs and TD using a Markov chain without memory, in which the transition to the next npLN-status depends only on the already achieved status. For each subgroup with npLNs there is a transition probability to the next subgroup with (n+1)pLNs. With only 12 probabilities, the 144 frequencies of Fig. 1a–b can be modeled and acceleration and duration of LN infiltration can be estimated. If the MET process is so easy to describe, given the appropriate function, then answers to the following questions should also be found: When and how are pLNs and METs initiated? How long are the growth periods of PTs, pLNs and METs? How do successful therapies work and can pLNs also initiate METs? Our analysis of over 30 thousand patients with HR positive BC presents the following 5 observations.

Firstly, *LN-infiltration* is a dynamic process in which the frequencies of patients diagnosed with a defined number of increasing pLNs, and thus the time spent in each subsequent pLN subgroup becomes smaller and smaller as the PT grows (Fig. 1a). With pT2 (mean 28 mm TD), the proportion of patients with a status of 3pLNs has dropped to 1/3 of that with 1pLN (Fig. 1a).

What could this accelerated infiltration cause? Four scenarios are conceivable. A long held perspective proposes that each pLN can infiltrate the LN-network in a cascade-like pattern. If this were the case, then an exponential increase in pLNs would be observed. This is not true: molecular studies have demonstrated that the gene signature of different pLNs can already be assigned to different areas in the causative PT [6]. Secondly, an increasing aggressiveness of disseminated TC can be dismissed. The infiltration process e.g. of the first LN in the remaining 0pLN subgroup is largely independent of the TD even if large PTs have already infiltrated many LNs. As the number of pLN increases, the impact on mortality is less dramatic (Fig. 2a). Third, a pre-metastatic conditioning of the LN microenvironment for homing of TCs has been discussed [7]. This too is not plausible, because an exponential increase of pLNs would also be expected. Thus, we propose the fourth scenario: the PT is the cause of both the acceleration in infiltration and in mortality because of an increasing number of disseminated MET-competent TCs. A PT

Table 1
Uni- and multivariate Cox regression for overall survival in the subgroup of invasive, not advanced and HR positive BCs (n = 37.265). CI: confidenz interval, HR: hazard ratio, pLN: positive lymph node.

Variable		univariate		multivariate		
	%	HR	p	HR	95% CI	p
Age: <50 years	21.4	ref	<.0001			<.0001
50 - < 70 years	53.3	1.36		1.47	1.38–1.57	
≥70 years	25.3	5.08		4.88	4.56–5.23	
TD: ≤ 5 (pT1a)	3.5	ref	<.0001			<.0001
5 - ≤ 10 (pT1b)	15.1	1.17		1.13	0.92–1.39	
10 - ≤ 20 (pT1c)	41.7	1.97		1.60	1.32–1.94	
20 - ≤ 50 (pT2)	33.1	3.90		2.37	1.95–2.87	
>50 (pT3-4)	6.6	8.62		3.43	2.81–4.19	
Grade: G1-2	77.8	ref	<.0001			
G3	22.2	1.71		1.32	1.26–1.39	<.0001
Number: 0 pLNs	64.8	ref	<.0001			<.0001
1 pLNs	13.0	1.43		1.26	1.18–1.35	
2 pLNs	6.1	1.67		1.43	1.31–1.55	
3 pLNs	3.4	1.95		1.54	1.39–1.70	
4-5 pLNs	3.9	2.35		1.78	1.62–1.95	
6-7 pLNs	2.3	3.18		2.27	2.04–2.52	
8-10 pLNs	2.3	3.78		2.55	2.30–2.52	
11-14 pLNs	1.8	4.53		3.07	2.75–3.42	
15-18 pLNs	1.1	4.56		2.95	2.57–3.38	
≥19 pLNs	1.3	5.60		3.60	3.19–4.06	

with 20 mm TD has about 8.5 billion TCs. At some point, the first MET-competent TC will emerge, through which the first TC will be disseminated and will be followed by more and more TCs from an expanding subarea after many duplications [8].

This leads us to the second major observation, the *initiation of life-threatening METs* and the sequential LN-infiltration should be analysed in tandem. The 20-year relative survival shows that about 17% of deaths are to be expected in the subgroup without pLNs (Fig. 2a). These are historical data and do not adequately reflect the success of adjuvant therapies. But they show that LNs and METs are initiated in parallel [9] and can remain occult for a long time (Fig. 2c). If a BC is already diagnosed with 1pLN, the mortality rate almost doubles. This symmetry suggests that there is a similar phase in the 0pLN-status where a PT can already disseminate MET-competent TCs and initiate the first MET.

In Fig. 1b this phase is designated as 0pLN*, in which MET-competent TCs are disseminated and isolated TCs or micro-METs can grow in LNs and organs [10–12]. The fraction of 0pLN* at 10/15/20/30 mm TD is 10.7/17.0/23.4/34.2%, respectively, and estimates the risk of MET in 0pLN patients (Fig. 1b). A comparable TD dependent risk is provided with recurrence scores, in particular when the increase in unfavorable clinicopathologic factors with TD is taken into account [13,14]. In our data, the 15-year cancer-specific mortality for pT1c/T2/T3-N0 is 7.4/20.8/38%, respectively, an increase, which is also shown by the Cox model for the pT-category.

In diagnosed subgroups with multiple pLNs, the first MET can also be initiated very early and then grow parallel to the PT. Due to the early initiation, the MET-free survival time is short. In 8–10 pLNs, already 13% of patients had primary advanced BCs (Figs. 2d) and 38% (25% at 5ys/65% at 20 ys, Fig. 2a) of non-advanced BCs died from tumor-related causes within 5 years after diagnosis. This sequential MET initiation is demonstrated by current genome analyses. With growing BCs, new mutations occur after each cell division and PTs become more heterogeneous [15–17]. Therefore, the MET of smaller BCs are genetically more similar to their PT than large BCs whose METs have grown longer in parallel with their PT [18].

MET is an autonomous process which shows survival after METs. The impact of the prognosis-relevant pLN-status on post MET survival is low. Tumors with many pLNs are not more aggressive, e.g. in comparison to HR negative BCs (Fig. 2b). This homogeneous growth also reflects the MET-free survival times (Fig. 2c). For small BCs, METs are initiated shortly before diagnosis, the growth time before BC diagnosis is short, and afterwards long. For large BCs the growth time of METs before BC diagnosis is longer and shorter thereafter.

Third, *growth durations* of tumor foci are required for interpreting the relationships of initiation, diagnosis and survival. The growth times of PTs from 10 to 20 mm were estimated using mammography screening data and yield a variation of 65/143/308 days for each volume doubling in the 25%/50%/75% percentiles, respectively [19]. With these estimations, a 2.5 mm PT reaches 28 mm after 10.5 vol doublings in 1.9/4.1/8.8 years. The TD represents the growth time in the Gfs.

Arithmetically, starting from the 1st cell of a PT, 34.3 vol doublings or 6.1/13.4/28.9 years for the 25/50/75 percentiles are required to attain up to 28 mm TD. Chemoprevention studies support these long growth periods with equally long-lasting effects after the end of chemoprevention [20]. For the growth time of MET, the twofold median MET-free survival time in the subgroup 0pLN (Fig. 2c) is an estimate and gives about 2 times 4 years (Fig. 2c) [21]. Extremes to median growth are only prior to diagnosis in T-N-M1 BCs or almost only after diagnosis in small BCs. Tumor foci in LNs are generally expected to grow faster. If a PT grows from 2.5 to 7.5 mm in 1.9 years and a tumor focus of 0.2 mm in an LN (pNmic) grows to 5 mm in parallel with the PT, pLNs would grow twice as fast as MET [22].

Fourth, *adjuvant therapies* over the past few decades have improved absolute survival by more than 20% [5,23]. Relatively, the proportion of cured patients decreases with increasing TD from about 90% to 25%. It is noteworthy that the absolute mortality reduction is largely independent of the TD [24]. LN and the parallel MET infiltration explain this. Evident METs are incurable, larger not-yet detectable MET must also be because detectability does not change the property of METs. But smaller MET are partially eradicable. Because there are smaller, undetectable METs in the subgroup 0pLN* as well as in those with few pLNs, and their distribution is largely independent of TD, this explains the identical decline in mortality for all larger PTs. For BCs <10 mm the proportions of 1–3 pLNs are still too small for a 20% improvement of survival. It follows that there are resistances already within the smallest METs initiated during 0pLN* [25].

Fifth, with regards to the *cause of the MET*, first of all the timing of the MET initiation has to be considered. Dormant TCs may be a life-long risk of MET initiation [26], yet this is partially refuted because many systemic therapies have been shortened without any disadvantages, i.e. no eradicable METs are initiated in the shortened phase [27,28]. Late initiations after a long MET-free interval are also unlikely, because otherwise there would have to be many triple negative BCs with fast-growing late METs (Fig. 2c). In all 0pLN BCs, about 20% MET occurs after 10 years, and favorable prognostic factors dominate. Among the pLN subgroups depicted in Fig. 2a, most METs – about 41% are observed at 0pLN and must be caused by PTs. PTs are also the cause for primarily advanced BCs, which increase with the number of pLNs (Fig. 2d). Therefore, no clinically relevant new METs will be initiated after R0 resections.

Survival as a function of TD in Fig. 1b also gives no indication of LN-initiated METs. Mean and number of all pLNs increase with the TD. The number increases in 100 patients, e.g. from 30 to 60 mm TD from 206 to 463 pLNs. If any pLN could initiate further METs, the survivor curve could not conform with the regression for >0pLN. The mortality would have to increase because every pLN is initiated by MET-competent TC, so that even a pLN could have more such TCs than the MET-competent area in the PT. Such an increase in risk is not observed. The lack of MET risk from pLN has now been confirmed in more than 10 tumor types by randomized studies

[29,30]. No single study has yet shown any benefit from lymph node dissection. Thus, pLNs cannot initiate METs in a clinically relevant manner, pLNs are dead ends for infiltrating TC [31,32]. This raises the question of the cause of successful radiation therapy of regional LNs [33].

This also means that even rare LN relapses are not life-threatening METs. The Sentinel concept is therefore a chance, not a risk [34]. However, pLNs can grow extracapsularly. This suggests control and selective removal in the aftercare. Radical LN dissections with many LNs are not justified for the improvement of the prognosis nor for staging. This does not rule out that MET-competent TCs can lymphogenously reach the circulation and then initiate METs.

Like the survival curves, the Cox model for overall survival shows the decreasing mortality risk with the increasing number of pLNs. Tumor size also contributes to survival and points to the different risk for 0pLNs, which is shown in Fig. 1b with the 0pLN*-status. The hazard ratios for the age of >70 years stand for the increasing risk of tumor-independent death.

Limitations of the analyses are to be considered. First, it is historical data from 3 decades. Neoadjuvant therapies reduce the available number of patients with pLNs in large tumors. Also the improved survival due to successful therapies in recent decades was previously mentioned. Second, there are missing values. Although they reduce the number of patients in multi-dimensional analyses, a selection of conspicuous disease courses has not yet been identified in any evaluations. Stage distributions and survival should therefore not affect the qualitatively relevant statements on the MET process, especially since the analyses were restricted to the HR positive BCs. The MET process is comparable to HR negative BCs in principle, only the growth of MET is about 2.5 times as fast (Fig. 2b). A strength of these analyses is that they show the importance of population-based, well-annotated real world data [35].

5. Conclusion

In summary, it follows from our analysis that LN-infiltration is a sequential and accelerating, but not hyper-complex process that does not change even with large BCs. Positive LNs are not a clinically relevant cause of MET, their numeric value is merely an indication of the duration of the dissemination of PTs and the risk of life-threatening MET that occurs in parallel with LN infiltration. It follows from genetic relationships between PTs and early and late initiated METs, as well as from studies on the lack of survival effects of LN dissections, that it is meanwhile common for all solid tumors, that pLNs do not initiate MET in terms of L.Weiss' 40-year-old metaphor, lymph node removal affects metastasis as well as removing the speedometer reduces the speed of a car [36]. Two challenges follow from LN-infiltration: How to improve survival in the low risk 0pLN-subgroup and why can only PTs and not pLNs metastasize?

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