N-terminal telopeptide (NTX) as a prognostic and predictive Marker of Bone Metastases in Breast Cancer patients after receiving bone supporting agents

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Abstract:

Background: Metastatic breast cancer (MBC), 60% of them will eventually develop bone metastases during their course of disease, N-terminal telopeptide is a telopeptide that can be used as a biomarker to measure the rate of bone turnover. Elevated levels of NTX are common in patients with osteolytic bone lesions, which is obviously in breast cancer. The aim of this study to demonstrate the affection of zoledronic acid and denosumab on serum NTX and on skeletal progression free survival.

Methods: The participants in this study were 81 bone metastatic breast cancer. Serum NTX levels as bone turnover marker was measured using the ELISA method baseline at start of the study and after 6 months of bone supporting agents receiving and compare between them.

Results: Significant reduction of serum NTX was observed in denosumab and zoledronic acid treatment at 6 months post treatment evaluation. According to skeletal PFS (progression free survival), a significantly relation present between NTX and PFS in both.

Conclusion: The present study demonstrated that decrease (at 6 months) of bone marker after introduction of zoledronic acid or denosumab is strongly prognostic.

KEY WORDS: Metastatic breast cancer, Bone metastases, Zoledronic acid, Denosumab, N-terminal telopeptide.

Introduction:

Breast cancer is the most common malignancy in women in the United States and is second to lung cancer as a cause death. The American Cancer Society has estimated that 279,100 Americans diagnosed with breast cancer and 42,690 die of disease in the United States in 2020 [1]. The most common sites of distant metastasis include bones, lungs, liver, and brain [2].

Bone metastases are common in metastatic breast cancer; bone is affected in more than 70% of patients with MBC [3]. It not only considerably reduces the OS but also the health-related quality of life due to pain, fatigue, and skeletal-related events (SREs) [4]. The currently available therapeutic strategies include a combination of the systemic therapies used in breast cancer (e.g., chemotherapy, ET, radiotherapy) [5] and those specifically targeting the bone, known as bone-modifying agents [6], Bisphosphonates and RANK/RANKL inhibitors represent the best agents for

the clinical management of patients with bone metastasis [7].

Bisphosphonates have a dual role in decreasing bone resorption by exerting an apoptotic effect on osteoclasts and increasing mineralization by inhibiting osteoclast activity [8], Zoledronic acid is a nitrogen-containing bisphosphonate and potent osteoclast inhibitor. The administration of these agents may reduce the risk of SREs and skeletal morbidity rate [9], Denosumab is a monoclonal antibody, targets the receptor activator of nuclear factor-kappa B (RANK) ligand. This drug inhibits the RANKL/RANK signaling mediated bone resorption, suppressing bone turnover and leading to the reduction of SRE risk [10].

Zoledronic acid is infused over a minimum of 15 minutes, and it is withheld if creatinine rises to further reduce the risk of renal injury, per zoledronic acid prescribing information [11]. Additionally, rapid normalization of elevated NTX levels during ZOL therapy has been associated with improved survival versus persistently elevated NTX levels [12]. This is a remarkable finding, as NTX is currently one of the most widely used markers to evaluate bone response to treatment in bone metastasis patients [13,14,15]. Elevated serum levels of NTX in the majority of patients with bone metastases can be normalized within 3 months of treatment of NTX after treatment with zoledronic acid have a similar prognosis as those with a normal pretreatment NTX level, but a longer progression-free survival than those still with higher NTX levels after treatment [13, 14].

Aim of the study:

Primary end point: The objective of our prospective study is to demonstrate the affection of zoledronic acid and denosumab on serum NTX levels and correlation between it and other factors in patient and disease criteria. Secondary end point: skeletal progression free survival analysis in both bone targeting agents in relation to NTX marker.

Patients and Methods:

Patients:

81 female of breast cancer patients with radiological evidence of newly diagnosed bone metastases admitted to our medical oncology department in South Egypt Cancer Institute, Assiut University. Eligible criteria of them were age ≥18 years old with histologically confirmed breast adenocarcinoma, recent radiographic (bone scan, or magnetic resonance imaging) evidence of bone metastasis, all sign an informed consent (Informed consent will be signed at the time of enrollment and prior to the collection of any specimens and or/clinical data). Exclusion criteria include patients having more than one cancer (second primary malignancy) pregnant and also patients with serious concomitant disorders that would compromise the patients ability to complete the study.

Study design:

Prospective, single center trial will be carried out in Medical Oncology department, South Egypt Cancer Institute, Assiut University, starting from 2019 until 81 patients are completely fulfilled. Patients were randomly assigned to receive either an intravenous infusion on 15 minutes of zoledronic acid 4 mg (group 1: N=41), Or a subcutaneous injection of denosumab 120 mg every 4 weeks (group 2:N=40). All regimens will be received under normal renal function tests and normal calcium level.

All patients included in this study will subject to baseline evaluation with full history taking, complete clinical examination, stage determination, complete laboratory investigations (complete blood count, liver function test, and renal function test, calcium level), imaging studies (CXR, Abdominal ultrasound, Bilateral Sonomammography) and bone scan and local MRI on boney metastatic site.

Follow up evaluation after 6 months on bone supporting agents with Clinical evaluation included assessment of performance status according to the World Health Organization (WHO) criteria, bone pain evaluation according to the Radiation Therapy Oncology Group (RTOG) pain score scale and recording of concomitant treatments (analgesics, anti-cancer therapy). Skeletal-related events, including pathological fractures, hypercalcemia, neurologic abnormalities due to spinal cord compression and need for bone irradiation, were also recorded. Bone scan and MRI on bonely metastatic site and the response interpreted according to the response evaluation criteria in solid tumors (RECIST) criteria.

Biochemical analysis:

Human N terminal type-1 collagen (NTX）ELISA kit used to assay the N terminal type-1 collagen (NTX) in the sample of human's serum

Test principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human N terminal type-1 collagen （NTX） in samples. Add N terminal type-1 collagen （NTX） to monoclonal antibody Enzyme well which is pre-coated with Human N terminal type-1 collagen （NTX）monoclonal antibody, incubation; then, add N terminal type-1 collagen （NTX）antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. then add Chromogen Solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concenthumanion of the Human Substance N terminal type-1 collagen (NTX) of sample were positively correlated.

Statistical Analysis:

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc.,

Chicago, IL, USA) version 21. Data were statistically described in terms of mean ± standard deviation (±SD), or median and range when not normally distributed, frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of quantitative variables was done using Mann Whitney U test because the data were not normally distributed. Comparison of paired quantitative variables was done by Wilcoxon signed rank test because the data were not normally distributed. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Odds ratio (OR) with 95% Confidence Interval (CI) and Logistic Regression was calculated to measure the different independent factors. Kaplan-Meier's method with log rank test, Cox regression method for univariate or multivariate overall and progression free survival analysis were used to assess the associations among different clinicopathological indices and patients outcome. P-value is always 2 tailed set significant at 0.05 level.

Results:

Baseline characteristics of BC cases according to the bone targeting agent received (n=81):

Cases classified into 2 groups: group 1: include 41 patients received intravenous zoledronic acid 4mg every 4 weeks on 15 minute infusion for six months. group 2: include 40 patients received subcutaneous denosumab 120 mg every 4 weeks for six months.

The demographic characteristics of the enrolled patients in Table (1). Showed that the Mean (± SD) age of patients received zoledronic acid was 51.05±10.87 years versus 49.95±12.53 years for those received denosumab with no statistically significant difference between them (P value = 0.67). And as regard menstrual state, 53.7% of the patients of zoledronic acid group were menopausal while 55.0% of the patients were postmenopausal in denosumab group with no statistically significant difference between them (P value =0.90). Left sided breast cancer represent the most common side in both groups as represent 61% in zoledronic acid group and 52.5% in denosumab group. Also MRM represent the most common type of surgery that done in both groups and IDC represent the most common post-operative histology in both groups with no statistically significant difference between them in these criteria (P value >0.05).

According to lymph node status, lymph node positive represent the most common finding in post-operative pathology, 87.8% of zoledronic acid group patients and 97.5% of denosumab group patients. Ki67 ≥15% represent the most common finding in postoperative pathology, 80.5% in zoledronic acid group patients and 97.5% of denosumab group patients.

According to sites of metastases, both bone and visceral metastases represent the most common finding in baseline imaging done, 61% of zoledronic acid group patients and 52.5% of denosumab group patients. Also in baseline bone scan finding to evaluate number of involved sites with bone metastasis, one site involvement was the commonest affection with metastases in zoledronic acid group patients (61%) versus more than one site boney affection was the commonest affection in denosumab group patients (55%). with no statistically significant difference between them in these criteria (P value >0.05).

All patients received chemotherapy or hormonal therapy as a palliative treatment with no significant differences observed between the 2 arms in the use of anti-tumor agents.

For accurate evaluation NTX value and its correlation with other variables, taken 0.84 nM BCE as cutoff value (Mean ± 2 SD of controls), by taken 60 patients with early breast cancer (EBC) represented in our study as control group, then compared them with our patient group study (81 patients with boney metastatic BC) that represented as cases, NTX levels measured in both arms by nM BCE (nanomole of bone collagen equivalent) and correlate to each other.

Then our Patients were divided into two groups for comparison with clinical outcomes: group with high expression NTX (above cutoff value) and other with low expression NTX (below cutoff value). In our study, found that the high expression is the most common findings in groups, 65.9% of zoledronic acid group and 67.5% of denosumab group patients.

Baseline and post-treatment NTX levels with zoledronic acid or denosumab:

At 6 months post treatment evaluation in zoledonic acid group, mean of NTX levels were significantly reduced from 3.62±4.67 reach to 0.58±0.49 as result to treatment (P value: 0.000). Also further significant reduction of the mean of NTX was observed in denosumab group at 6 months post treatment evaluation, reduced from 3.75±4.72 reach to 0.86±0.53 as result to treatment (P value: 0.000), concluded statistically significant reduction in serum NTX level after zoledronic acid treatment and denosumb treatment. but with no any statistically significance between both groups in baseline or after 6 months of treatment (P value: 0.906) (P value: 0.112) respectively. The present study demonstrated that decrease at 6 months of bone marker after introduction of zoledronic acid or denosumab is strongly prognostic.

Skeletal progression survival analysis according to the NTX tumor biomarker (n=81):

In analysis of skeletal progression free survival according to NTx tumor marker at 1 year, found that 59% of low expression group of patients have no any skeletal progression versus only 12% of high expression presented with no skeletal progression (mean that 88% develop progression in patients with high NTX values).

Univariate analysis of 81 patients with BC according to clinic-pathological variables:

According to PFS (progression free survival): The probability that patients with Grade (1or 2) to showing progression are 60% lower than patients with Grade (3 or 4) (95%CI 0.206-0.792). (P value: 0.008) mean that there is significantly relation. Also the probability that

patients with low NTx expression to showing progression are 65% lower than patients with high NTX expression (95%CI 0.145-0.835). (P value :0.008) mean that there is significantly relation.

According to Response (Regression + Stationary disease): Patients with Grade (1or 2) are about four times more likely to achieve response than patients with Grade (3 or 4) (95% CI 1.275-11.026). (P value: 0.016). Also Patients with smaller tumor size (T1or T2) are about three times more likely to achieve response than patients with lager tumor size (T3 or T4) (95%CI 1.051-6.374). (P value: 0.039). according to NTX levels, Patients with low NTX expression are about five times and half more likely to achieve response than patients with high NTX expression (95%CI 1.906-15.867). (P value: 0.002)**.**

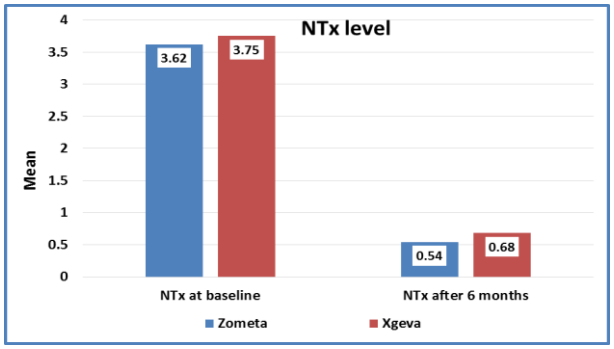

Figure (1): Bar graphs showing the difference of NTx tumor biomarker level from baseline to after 6 months of treatment according to bone targeting agent

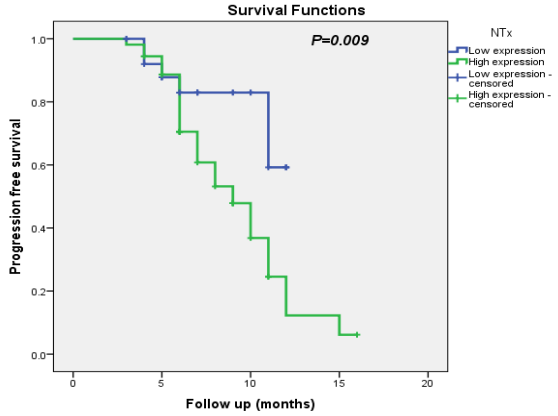

Figure (2): Kaplan-Meier's curve showing skeletal PFS according to NTX level

Discussion:

N-terminal telopeptide (or more formally, amino terminal collagen crosslinks, and known by the acronym NTX) is a telopeptide that can be used as a biomarker to measure the rate of bone turnover. NTX can be measured in the urine (uNTX) or serum (serum NTX) [16]. NTX decreases with the use of bone targeting agents [17]. Both baseline and serial NTX levels after BTA (bone targeting agents) therapy are strongly prognostic [18].

We conducted a Prospective cohort study of 81 patients who received bone supporting agents for stage IV breast cancer cases with bone metastases at Medical Oncology department of SECI, Assuit University starting from 2019 until 81 patients are completely fulfilled

In evaluation of response in bone metastases according to NTX values found that NTX level differ significantly with response of bone disease (P value: 0.002), the largest percentage of regression or remained stable disease (77%) mostly associated with low expression NTX values, and the rate of bone disease progression was significantly higher in patients with NTX high expression(63%) compared to those with NTX low expression (22.2%) at post-treatment evaluation .These results are agree with other authors, suggest that the bone resorption marker, NTX, is associated with the presence and extent of metastases, response to treatment, and prognosis [19,20, 21].

In analysis of skeletal progression free survival according to NTx tumor marker at 1 year, found that 59% of low expression group of patients have no any skeletal progression versus only 12% of high expression presented with skeletal progression.

Increased levels of NTX predicted a number of negative outcomes, including skeletal-related events, disease progression and death as In Univariate analysis of patients with BC according to clinic-pathological variables: results showed the probability that patients with low NTx expression to showing progression are 65% lower than patients with high NTx expression (95%CI 0.145-0.835). in agree with Coleman RE et al, larger post hoc analysis of patients with bone metastases from breast cancer, prostate cancer, lung cancer, or other solid tumors or bone lesions from multiple myeloma [13] results showed that elevated NTX levels were associated with a significant 2-fold increased risk of disease progression and risk of skeletal complications (P < .001 for all)[13].

The major finding of our study was the predictive value of NTX on treatment strategies. The level of NTX that significantly decreased in both arms of our study after bone supporting agents (with zoledronic acid and denosumab) mean decreased reduction of bone mineral density that mostly occur at the result of bone metastases in breast cancer disagree with other trials even observed an increase of bone mineral density in patients receiving bisphosphonates [22,23].

Table (1) Baseline characteristics of BC cases according to the bone targeting agent received (n=81).

Variable name	Zometa (n=41)		Xgeva (n=40)		*P value*
Age (years), mean±SD	51.05±10.87		49.95±12.53		0.674
Median (range)	50 (25 – 73)		50 (25 – 75)		
Menopausal status					0.904
• Pre-menopausal	19	(46.3)	18	(45.0)	
• Post-menopausal	22	(53.7)	22	(55.0)	
Tumor laterality					0.563
• Right	14	(34.1)	18	(45.0)	
• left	25	(61.0)	21	(52.5)	
• Bilateral	2	(4.9)	1	(2.5)	
Type of surgery					0.821
• MRM	26	(63.4)	27	(67.5)	
• BCS	4	(9.8)	2	(5.0)	
• No surgery	11	(26.8)	11	(27.5)	
Pathological type					0.261
• IDC	32	(78.0)	39	(97.5)	
• ILC	9	(22.0)	1	(2.5)	
Tumor grade					0.749
• Grade I, II	31	(75.6)	29	(72.5)	
• Grade III, V	10	(24.4)	11	(27.5)	
Tumor size					0.921
• T1-T2	23	(56.1)	22	(55.0)	
• T3-T24	18	(43.9)	18	(45.0)	
Lymph node status					0.201
• Negative	5	(12.2)	1	(2.5)	
• Positive	36	(87.8)	39	(97.5)	
Luminal A					0.565
• No	19	(46.3)	16	(40.0)	
• Yes	22	(53.7)	24	(60.0)	

Luminal B					0.712
• No	38	(92.7)	36	(90.0)	
• Yes	3	(7.3)	4	(10.0)	
Her2neu overexpression					0.494
• No	41	(100.0)	39	(97.5)	
• Yes	0	(0.0)	1	(2.5)	
Triple negative					0.271
• No	25	(61.0)	29	(72.5)	
• Yes	16	(39.0)	11	(27.5)	
Ki67 (%)					0.228
• <15	8	(19.5)	1	(2.5)	
• ≥15	33	(80.5)	39	(97.5)	
Site of Metastasis					0.441
• Bone only	16	(39.0)	19	(47.5)	
• Bone + Visceral	25	(61.0)	21	(52.5)	
Baseline bone scan					0.150
• One site	25	(61.0)	18	(45.0)	
• > one site	16	(39.0)	22	(55.0)	
Baseline NTX tumor biomarker					0.875
• Low expression	14	(34.1)	13	(32.5)	
• High expression	27	(65.9)	27	(67.5)	

Quantitative data are presented as mean ± SD or median (range), qualitative data are presented as n (%). Significance defined by $p < 0.05$. Luminal A: [ER+/PR+, HER2-], Luminal B: Triple positive.
(Ki67 percentage calculated as last guideline update at date of study to start, National Comprehensive Cancer Network version 2019.)

Table (2): NTX tumor marker before and after treatment by bone targeting agent (n=81) by nM BCE:

NTx level (nM BCE)	**Zometa (n=41)**	**Xgeva (n=40)**	***P value*[1]**
At baseline			0.906
Mean ± SD	3.62±4.67	3.75±4.72	
Median (range)	1.3 (0.4 – 17.9)	1.4 (0.1 – 16.6)	
After 6 months			0.112
Mean ± SD	0.54±0.45	0.68±0.43	
Median (range)	0.4 (0.1 – 1.8)	0.7 (0.1 – 1.7)	
***P value*[2]**	**0.000***	**0.000***	

Quantitative data are presented as mean ± SD and median (range). Significance defined by $p < 0.05$.
P value[1]: comparing both studied groups.
P value[2]: comparing the same group from before to after treatment.

Table (3): Skeletal Progression free survival analysis according to NTx tumor biomarker

NTX	Median				Log Rank (Mantel-Cox) p-value
	Estimate	Std. Error	95% Confidence Interval		
Low expression	9.000	0.953	7.132	10.868	
High expression	10.000	0.588	8.847	11.153	**0.009***

Skeletal PFS	Estimate ± SE		value
	Low expression	High expression	
At 1 year	59.2±15.2%	12.3±7.3%	**0.009***

Table (4): Univariate analysis of 81 patients with BC according to clinic-pathological variables:

	PFS			Response (Remission + SD)		
Variable name	**p-value**	**HR**	**95% C.I. for HR**	**p-value**	**OR**	**95% C.I. for OR**
Age	0.192	1.021	0.990-1.053	0.588	0.990	0.953-1.028
Tumor grade	**0.008***	0.404	0.206-0.792	**0.016***	3.750	1.275-11.026
Tumor size	0.228	0.674	0.355-1.279	**0.039***	2.588	1.051-6.374
NTX	**0.018***	0.348	0.145-0.835	**0.002***	5.500	1.906-15.867

In comparing denosumab, a fully human monoclonal antibody against receptor activator of nuclear factor _ B (RANK) ligand, with zoledronic acid in delaying or preventing skeletal-related events (SREs) in patients with breast cancer with bone metastases. In other words, In evaluation of skeletal progression free survival at 1year, found that 30.5% of patients are progressed in zoledronic acid group that closely near to percentage of denosumab group which 30.2%. So, there was no significant difference between both groups in corresponding Kaplan-Meier curves (p value :0.119) that accordance with Lipton et al, Brown et.al and Body et al, concluded that denosumab has also been found to be effective in delaying SREs in advanced cancer. As a human monoclonal antibody, denosumab binds to receptor activator of nuclear factor kappa-B ligand (RANKL) (Lipton A et.al., 2010) (Brown JE et.al.,2012) (Body JJ et.al.,2012) and has been shown non-inferior to ZA.

Given the prompt response of bone markers to treatment with bone supporting agents and their use as predictors of their efficacy (zoledronic acid or denosumab), We hypothesized that the serial measurement of bone markers could be a strategy to tailor therapy regimen. By evaluation of Baseline and post-treatment with zoledronic acid or denosumab NTX level, serum NTX levels are significantly decrease in patients with bone metastases after bone supporting agents treatment regularly. In agreement with Some authors as Clemons et al, Clemons MJ et al and Pectasides et al that have shown that high urinary or serum NTX levels should decrease after bisphosphonate treatment, as a sign of the response to antiresorptive treatment [27, 28]. Therefore, serum NTX evaluation is potentially useful for following up of patients with BMs who are treated with these antiresorptive agents, also in agreement with Mercatali et al that concluded, In the panel of markers investigated, only NTX exhibited a significant change over time, decreasing by 26% with respect to baseline levels ($P<0.0001$) in patients received 4 mg of ZA over 15 min as an intravenous infusion every 28 days. which were conducted every 3 or 4 months after the diagnosis of bone metastases for a maximum of 12 months [29].

Brown et al concluded that in patients with known bone metastases from solid tumors, increases in bone ALP or NTX predicted increased rates of skeletal-related events, such as fracture, disease progression or death [14] .Furthermore, normalization of NTX levels after treatment was also correlated with a longer event-free and overall survival when examined across several studies of solid tumors, suggesting that bone targeting agents may have utility in monitoring therapy in this setting [12] that in agree with our study.

Conclusion:

NTX is an important biochemical marker of bone turnover. Denosumab was not superior to zoledronic acid for delaying or preventing SREs but has several potentially beneficial characteristics for patients. Also decrease (at 6 months) of bone marker after introduction of zoledronic acid or denosumab is strongly prognostic.

Abbreviations

ALP	Alkaline phosphatase
BC	Breast cancer
BCS	Breast conservative surgery
BTA	Bone targeting agents
CI	Confidence interval
CXR	Chest x-ray
EBC	Early breast cancer
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Estrogen Receptor
HER2	Human Epidermal Growth Factor Receptor 2
IDC	Invasive ductal carcinoma
ILC	Invasive lobular carcinoma
MBC	Metastatic breast cancer
MRI	Magnetic resonance imaging
MRM	Modified radical mastectomy
nM BCE	Nanomole of bone collagen equivalent
NTX	N-terminal telopeptide
OR	Odds ratio
OS	Overall survival
PFS	Progression free survival
PR	Progesterone Receptor
RANK	receptor activator of nuclear factor-kappa B
RANKL	receptor activator of nuclear factor-kappa B ligand
RECIST	Response Evaluation Criteria in Solid Tumors
RTOG	Radiation therapy oncology group
SD	Standard deviation
SECI	South Egypt Cancer Institute
SPSS	Statistical package for the social science
SREs	Skeletal related events
WHO	World health organization
ZA	Zoledronic acid
ZOL	Zoledronic acid

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Nil.

Conflicts of interest

There are no conflicts of interest.

Authors' contributions

This work was carried out in collaboration among all authors. Authors Adel Gabr, Ashraf Z. Abdalla, Eman M. Zaki and Abdallah H. Mohammed are designed the study, performed the statistical analysis, and wrote the protocol. Author Shaimaa A. Badawy collected the patients' data and wrote the final draft.

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