Original Article

Epidemiological and survival analysis of triple-negative breast cancer cases in a retrospective multicenter study

Sarin R, Khandrika L[1], Hanitha RNM[1], Avula A[1], Batra M[1], Kaul S, Raj H[2], Shivkumar S[3], Gupta S[4], Khan E[4], Bhandari TPS[5], Prasad SVSS[5], Reddy VA[5], Swarnalata G[5], Bakre M[6], Chatterjee S, Jain J

Indraprastha Apollo Hospitals, New Delhi, [1]Sapien Biosciences Pvt. Ltd., [5]Apollo Hospital Enterprises, Hyderabad, Telangana, [2]Apollo Speciality Hospital, Chennai, Tamil Nadu, [3]Apollo Hospital Enterprises, [6]OncoStem Diagnostics Pvt. Ltd., Bengaluru, Karnataka, [4]Apollo Gleneagles Hospitals, Kolkata, West Bengal, India

Correspondence to: Dr. Jain J, E-mail: jugnu@sapienbio.com

Abstract

INTRODUCTION: This is a retrospective study with data collected from breast cancer cases from five major Apollo Hospitals across India, as part of a biobanking process. One aspect of our study focused specifically on data from triple-negative breast cancer (TNBC) cases. The aim of this study was to analyze epidemiology, treatment options, and survival of the patients with TNBC. Our goal was to draw conclusions on the preponderance of the disease and also to understand the outcomes using the existing therapy options. **MATERIALS AND METHODS:** Data were collected after due ethical clearances and were coded with regard to patient identifiers to protect patient privacy. Data were not only from the various departments of the respective hospitals and the treating physicians but also from the follow-up made by hospital staff and social workers. **RESULTS:** About 20% of all cases of breast cancer comprised TNBC. Although the disease is generally thought to be an early onset disease, there was no major difference in the median age of diagnosis of TNBC compared to other breast cancer cases. More than 85% of the TNBC cases were of early stage disease with <4% of the cases of metastatic cancer. Data on follow-up were somewhat sporadic as a good number of cases were lost to follow-up, but from the available data, recurrence rate was about 11%. Death, when it occurred, was mostly in the early periods of treatment with 35% of the events occurring before 3 years. The overall survival rates beyond 3 years were more than 86%. **CONCLUSIONS:** Data and sample collection are an ongoing process, so we expect this data set to be enriched with more cases and longer duration of follow-up in a year. Preliminary analysis sheds light on the potential of such a collection both for understanding the epidemiology of the disease and also for conducting future studies with an eye toward improving treatment outcomes.

Key Words: Biobank, multicenter study, retrospective study, treatment outcomes, triple-negative breast cancer

Introduction

Even though rapid advances have been made in the treatment of cancers, a subset of patients are still vulnerable due to the lack of or reduced response to therapy. Currently, the physician makes a decision on the choice of the therapy based on clinical, pathological, and a small number of molecular factors. However, the complexity of the disease makes it difficult to generalize the treatment choice and expect similar results in a vast number of patients. Response to a particular therapy may be a result of many factors some of which are the presence or absence of specific gene mutations, the stage of cancer, the hormonal status of the tumor, and many others which may not have been identified as of yet.

A detailed study into already existing data in terms of response to specific treatment paradigms with respect to the disease state, stage of the disease, time from diagnosis, use of targeted therapies, outcomes, etc., may in fact help in determining optimal treatment options in seemingly similar cases in the future. The value of such retrospective studies cannot be stressed enough.

Triple-negative breast cancer (TNBC) is still a challenge due to its heterogeneity in terms of the physiological behavior, the prognosis of the disease, and the difficulty in diagnosing it through the use of mammography.[1] Even though the disease may respond well to normal chemotherapy agents including anthracyclines (ACs), taxanes, and cyclophosphamide,[2] the disease-free survival and overall survival (OS) are still lower than the other types of breast cancer. Therapies that target the estrogen receptor (ER), progesterone receptor (PR), and Her2 receptors are of no use in TNBC making the long-term management of disease difficult.

Retrospective analysis of the treatment paradigms and the outcome of such treatments in conjunction with disease biology, may hold immense value in segregating patients into specific groups that may have a better response to traditional chemotherapy, based on specific clinical and pathological features. Such studies can also aid in the development of new predictive markers to better stratify patients.

To systematically study patient treatment and improve upon current treatment paradigms by the development and use of more effective therapies and/or diagnostic and theranostics markers, an institutional biobank, Sapien Biosciences, has been established. The biobank has retrieved a few years' worth of retrospective samples from several Apollo Hospitals along with the available patient medical records, the process is ongoing. The samples span different years due to each hospital's archival of the same. The data collected are for patients for whom left-over surgical samples are available at the biobank. Patients for whom surgery is not typically performed, for example, Stage 4, are therefore not captured in full, in this retrospective analysis.

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The aim of this study was to analyze the retrospective data collected as part of the biobanking process in the form of epidemiology, therapy options, and disease-free or OS of the patients from five major Apollo Hospitals. The five centers were from Bengaluru, Chennai, Delhi, Hyderabad, and Kolkata. Our goal was to not only draw conclusions regarding the occurrence, age of onset, and the pathology of the cases seen but also to understand the therapeutic regimens opted for the treatment and their effectiveness vis-a-vis global outcomes.

Materials and Methods

Patient samples and data acquisition

All data pertaining to this study were collected retrospectively. Appropriate clearances were obtained from the respective Hospital Ethics Committees. Patient identifier data were anonymized to preserve patient confidentiality.

The study spanned five major centers of Apollo across India. Data gathered consisted of epidemiology, demographics, treatment, and disease-free survival information. Due to the retrospective nature of the study, data collected varied with respect to time periods based on availability of the same at each hospital, i.e. Bengaluru from 2008 to 2012, Chennai from 1997 to 2011, Delhi from 2005 to 2012, Hyderabad from 2006 to 2013, and Kolkata being the smallest collection from 2009 to 2010. Data were collected and pieced together from multiple departments including medical records, hospital information database, histopathology, chemotherapy and radiation departments, treating oncologists, and social workers. Data available also varied from patient to patient and the time of follow-up was also different.

Immunohistochemical (IHC) staining for ER, PR, and Her2 status was performed by the Pathology Departments of the respective hospitals and was according to the standard practices. Survival data were analyzed using the GraphPad Prism 6 software (Graphpad software Inc., La Jolla, CA, USA).

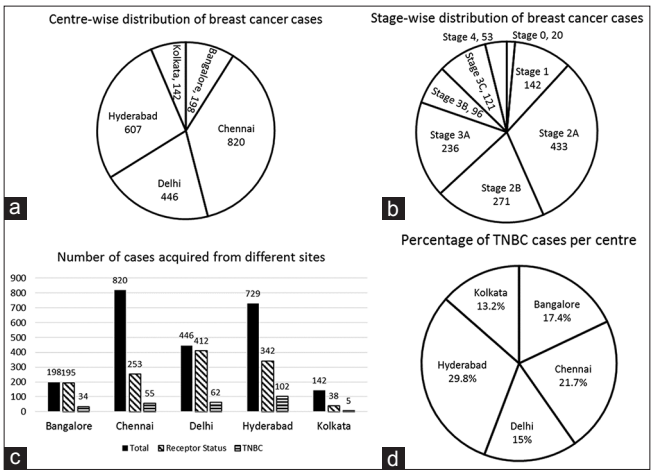

Figure 1: Distribution of total breast cancer cases based on (a) the site of collection, (b) the different stages of cancer found. (c) The number of cases for which the estrogen receptor, progesterone receptor, and Her2/neu staining status was known and the number of triple-negative breast cancer cases from each center. (d) Pie chart depicting the percentage of triple-negative breast cancer in the total cases from different sites

Results

Triple-negative breast cancer comprises about 20% of all Indian breast cancer cases combined

Data on the breast cancer cases referred to five of the major Apollo Hospitals were collected. Cancer centers at Chennai, Hyderabad, and Delhi contributed the most number of cases [Figure 1a], namely, 820, 607, and 446 respectively, due not only to the streamlined nature of the data acquisition system but also to the participation of some physicians in particular centers. Centers at Bengaluru and Kolkata contributed 198 and 142 cases which are a small percentage of the cases seen at the respective centers due to the paucity of data. More cases may be logged from these centers in future if data are collected prospectively.

Out of more than 2200 breast cancer cases studied, the staging information was available for only 62% of the cases. Where available results of our analysis show that there is a representation of all the different tumor-node-metastasis stages of breast cancer, with the Stage 0 being the lowest in number (1.45%) and the Stage 2a (31.5%) being the highest [Figure 1b].

One of the limitations of our collection was that the starting source of the data was the information on the tissue samples stored at the respective Pathology Departments postsurgery. This may have introduced an unintentional bias in the lack of number and distribution of cases that belong to the later stages, especially that of Stage 4, where surgery may not be the choice of treatment, and so tissue availability may not be a true reflection of the actual numbers of cases. The major source of information on Stage 4 cases in this study was through the physician's medical records.

Staining for the ER, PR, and the Her2 receptor is a means for opting for specific treatment. The data collected showed that the staining for the receptor status was not a standard procedure as of 2011, with 3 out of the 5 hospitals not having complete data on the receptor staining [Figure 1c]. In a majority of the cases, staining for Her2 was lacking, whereas ER and PR staining data were readily available. While Delhi and Bengaluru had data on more than 90% of the cases, Kolkata had the least amount of receptor status data (about 27%). Hyderabad and Chennai had the data for 47% and 31% of the available cases, respectively.

Out of the 1240 cases for which the ER, PR, and Her2 data were available, 257 (20.8%) of the cases were TNBC by IHC staining and fluorescence *in situ* hybridization [Figure 1d]. The percentage of TNBC varied between 13% and 22% of the overall breast cancer cases from four of the centers in this study, but the Hyderabad center had a higher percentage with nearly 30% of the cases reported TNBC.

No major difference in the mean age at diagnosis of triple-negative breast cancer compared to all breast cancer cases

TNBC is considered to be an early onset disease.[3] In our study population, we did not find any significant difference in the median age of TNBC occurrence compared to

total breast cancer, at diagnosis [Figure 2]. In the overall breast cancer cases across all the centers, the median age at diagnosis was more than 50 years with only a year or two lower in the case of TNBC. In the cases from Hyderabad, however, about 60% of TNBC cases were below 50 years of age, bringing the median age at diagnosis down to 46 years, in contrast to all other centers [Table 1]. The minimum age of presentation was 14, reported from the Chennai center and the maximum was 81, from Delhi center.

Most of the triple-negative breast cancer cases are of early stage cancers

Early breast cancer (Stages 1, 2a, 2b, and 3a) comprised about 85.4% of 257 cases in TNBC included in this study [Figure 3a], with Stage 2a being the most common (38.5%). Within Stage 2a, most of the cases (77%) were patients above 40 years of age [Table 2]. Convincingly, the age group comprising 51–60 years contained the most number of cases across all stages except for Stage 3a, where the highest number of cases was between the age group 41–50 years.

Evaluation of the stage-wise distribution across various age groups showed that the group below 30 years of age consisted of only 10 cases [Table 2] with stage information known for only 8 cases. The age group over 50 years consisted of 180 cases, the largest number out of 240 cases.

When compared within age groups, Stage 4 cancers constituted only 1% in the age group 31–50, whereas they were about 8% in the above 50 year age group [Figure 3b]. This number was surprisingly lower than expected as TNBC is considered to be a difficult disease to detect through regular mammography.[4] The highest number (16.4%) of late stage cancers (Stage 3b, 3c, and 4) was also seen in the over 50-year group, compared to 12.4% in the 31–50-year group. The low number of late stage cancers may not be a true representation of the frequency of occurrence as our collection was focused mainly on surgical tissues and surgery is not common for Stage 4 cancers.

For most breast cancers, the preferred treatment regimen consists of surgical removal of the cancer tissue followed by chemotherapy and/or radiotherapy. The type of surgery, either modified radical mastectomy (MRM), or breast conservation surgery (BCS), depends on a few factors such as the stage of cancer, the involvement of the lymph nodes, and the preference of the surgeon. Data on the type of surgery were available for 237 cases in which 181 opted for MRM. Our results suggest that the type of surgery depended on the surgeon's preference as exemplified by data from Chennai and Bengaluru centers [Figure 4a]. More than 95% of the surgeries performed were MRM in these centers; however, this data may be incomplete due to the number of participating physicians being lower from these two centers. Despite this, results showed a good correlation between the stage of cancer and the choice of surgery, with lumpectomy being confined only to early TNBC [Figure 4b]. For later stages of TNBC, MRM was the preferred surgery type. In the case of Stage 4 cancers, lumpectomy may have been offered as a palliative surgery.

Out of the 257 cases of TNBC, chemotherapy regimen data were available for 180 cases. Generally, depending on the aggressiveness of cancer, ACs alone or ACs followed by taxanes (T) are the preferred choice of first line of chemotherapy for all breast cancers. Data available showed

Table 1: Percentage of triple-negative breast cancer and all breast cancer cases below or above 50 years of age, at the time of diagnosis

	Total		TNBC	
	<50	>50	<50	>50
Bangalore	40.1	59.5	38.2	61.8
Chennai	46.6	53.4	49.1	50.9
Delhi	37.9	62.1	33.9	66.1
Hyderabad	46.4	53.6	65.7	33.3
Kolkata	50.7	49.3	20.0	80.0

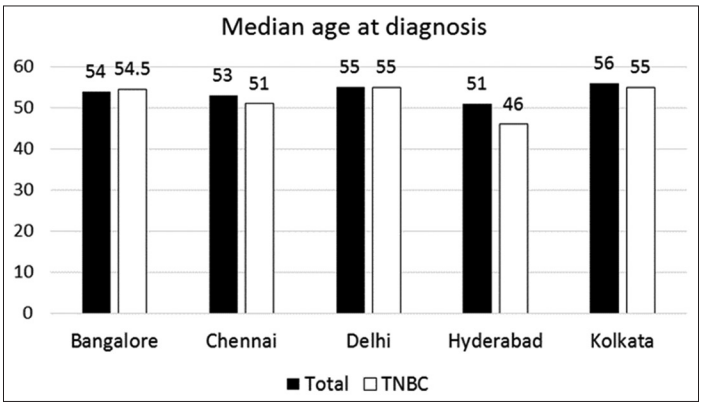

Figure 2: Median age, in total breast cancer cases and in triple-negative breast cancer, at diagnosis, from different centers

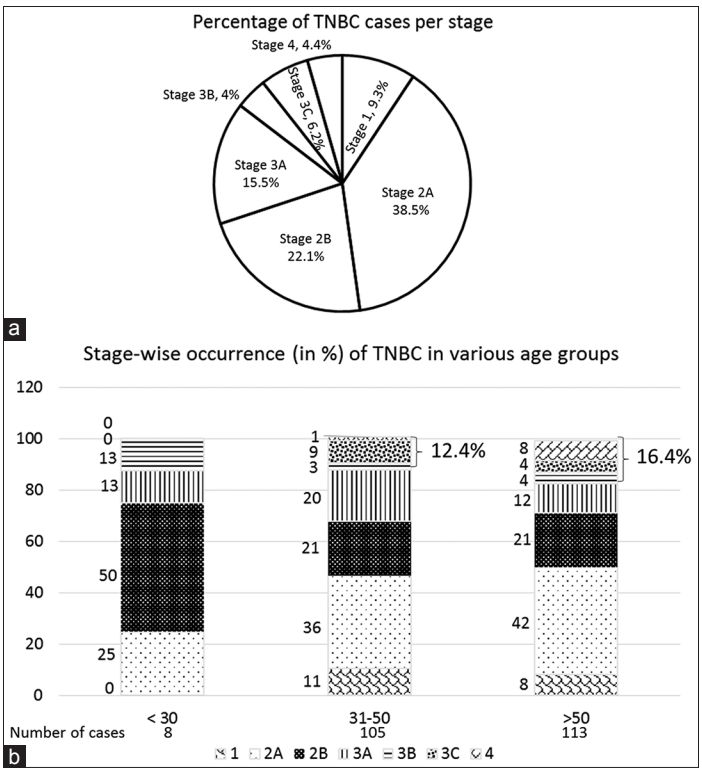

Figure 3: (a) Pie chart showing the different stages of the triple-negative breast cancer cases from all the centers. (b) Triple-negative breast cancer cases were sorted into three groups depending on the age at diagnosis and then further grouped by the stage of the cancer

that the choice of the first line of therapy was evenly distributed in the five centers, among the different therapies available [Figure 4c]. ACs alone were commonly used in cases with Stage 1 and Stage 2a TNBC (more than 60% of the cases), whereas in the rest of the cases, ACs followed by taxanes were more common [Figure 4d].

Survival

Of the 257 cases of TNBC, 102 cases had data on follow-up posttreatment. Serendipitously, most of the follow-up data available was for early breast cancer with only nine cases registered that were Stage 3b or higher. The minimum time for follow-up in this study was 1 month and the longest was more than 6 years; however, the median follow-up was 3 years after which many cases were lost to follow-up. Data presented in this study were based on the last follow-up data available rather than OS. Due to this, the survival data was presented until the last follow-up and need not always represent mortality.

Recurrence rate was relatively low with only 11.7% of the cases known to have recurrence [Table 3], compared to a similar study by Zhang *et al*.,[5] wherein the recurrence rate was 19%. Of the 12 known cases, 6 of the cases (85% of TNBC cases for which follow-up data were available in that year) showed recurrence within a year postsurgery. The rate of disease recurrence dropped to 33% by the 2nd year and further to 11% by the 3rd year.

In a follow-up period of 5 years, 23 breast cancer-related deaths were recorded. Out of these, 30% of deaths occurred between 2 and 3 years postsurgery. The OS rates beyond 3 years were encouraging (about 86%).

Data regarding the type of surgery and chemotherapy for each case were available for 86 of the cases, and 59 of those were from MRM group. Thirty-four out of the 59 MRM cases were treated with ACs alone and 25 were treated with a combination of ACs and taxanes [Figure 5a]. Stage 1 cases, which were only 8, showed 100% survival up to 2 years irrespective of the treatment regimen opted for but dropped to 12.5% by 5 years. The median survival for the MRM-AC arm was 48.5 months, for MRM-combination arm was 35.8 months, for the BCS-AC arm was 69.1 months, and for the BCS combination arm was 54.9 months.

The 3 year survival was the highest (100%) in the BCS ACs arm, whereas the lowest (48%) was seen in the MRM treated with ACs and taxanes combination arm. The 3-year survival for MRM ACs arm and for the BCS combination arm were about 80% and 78.9%, respectively. Twenty cases (23.2%) have survived longer than 5 years.

Irrespective of the type of treatment, Stage 2 cases had good 3 year survival rates, with 91% for Stage 2a and 80% for Stage 2b [Figure 5b]. Cases from Stage 3a, however, had a 3-year survival of only about 46% which was lower than the global average of 55%.[6]

Table 2: Distribution of triple-negative breast cancer cases based on the stage of the disease and age at diagnosis. Number of cases for which both the stage and age information was available were 226 out of a total of 257 cases, whereas the age at diagnosis was known for 240 cases

	Stage	1	2A	2B	3A	3B	3C	4	Not Known
Total stage		21	87	50	35	9	14	10	31
<20	0	0	0	0	0	0	0	0	0
21-30	10	0	2	4	1	1	0	0	2
31-40	50	8	18	12	5	1	3	1	4
41-50	65	3	20	10	16	2	6	0	10
51-60	63	8	24	12	9	3	4	4	12
61-70	43	1	19	11	3	2	1	3	3
>70	9	1	4	1	1	0	0	2	0
Total Age	240								
% (stage)		8.2	33.9	19.5	13.6	3.5	5.4	3.9	12.1

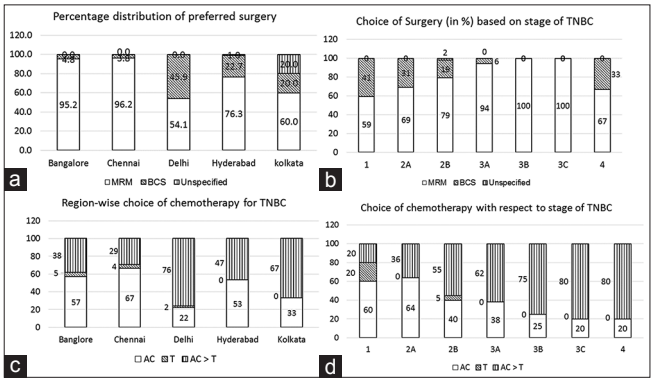

Figure 4: (a) The choice of surgery for triple-negative breast cancer among the centers and (b) the type of surgery based on the stage of cancer. (c) Chemotherapy used in each case was categorized as anthracycline-based, taxanes alone, or a combination of both (anthracycline > taxanes). Distribution of the choice of chemotherapy among the various centers is shown as a stacked bar graph. (d) Choice of the class of chemotherapy drugs with respect to the stage of the triple-negative breast cancer is shown

Discussion

TNBC is of serious concern due to its heterogeneity. The pathology of the disease may vary from a mild form to an aggressive disease with a high rate of recurrence. One of the major concerns with treating TNBC is the ineffectiveness of therapies targeted against the ER, PR, and Her2 receptors. Modern day therapies that are designed against the three receptors have shown great promise in treating those breast cancers. It is this distinct lack of any targeted therapies for TNBC that makes treating this cancer that much difficult. However, when detected early and treated adequately, the survival rates of TNBCs have been reported to be equal to those that can be achieved with targeted therapies in other breast cancer types.[7]

In this study, our objective was to understand the distribution of the TNBC cases across multiple centers located in different geographies. We focused mainly on the epidemiology, the type of surgery, and chemotherapy used to draw conclusions about how the differences in the type of treatment affected the survival of the patients. This was a retrospective study with the data collected over a period varying between 6 and 14 years in the different centers. While the data were not complete for every case, the total number of cases was large enough to enable drawing some indicative comparisons between this dataset and prior publications.

Previous reports from international groups have reported a significant difference in the occurrence of TNBC based on ethnicity. TNBC has been reported to have a higher rate of occurrence in Hispanics, Africans, and African-Americans (ranging from 25% to 60%) compared to Caucasians (12–16%).[8,9] Studies by Indian groups have reported a wide range of TNBCs from 11.8% to 31.9% in Indian population.[10,11] Our study shows an average of 20% TNBC across five centers. It was interesting to note that cases from Hyderabad had a higher incidence of TNBC compared to the other centers (Chennai, Bengaluru, Delhi, and Kolkata) which had comparatively similar incidence rates among them. There, however, was no clear-cut North-South geographical differentiation in the percentage of TNBC cases.

General consensus from both Indian and Western data suggested that TNBC is seen at an earlier age with the median age of presentation at least 5–10 years before the median age of other breast cancers. Our study did not show much difference in the median age of presentation, ranging from 51 to 56 years, between TNBC and total breast cancer cases. Only in the cases presented at Hyderabad was a 5-year difference with TNBC cases being presented earlier. Earlier studies involving Indian population showed great variation in the observed median age at diagnosis of TNBC. Ghosh *et al*.[12] reported that nearly 42% of TNBC cases were below the age of 35 years, whereas the mean age of presentation as reported by Suresh *et al*.[13] was 49 years, which was similar to that seen in our study (52.3 years). Interestingly, the age of presentation of TNBC in our study was similar to the age reported in a study from the Western population (53 years).[6]

Among the various stages of TNBC, Stage 2 was the highest represented among all the centers (about 62%) followed by Stage 3 (about 20%). It was surprising that the Stage 4 cancers formed only 4.4% of the total TNBC cases in our study. However, this could be an unintentional bias introduced due to the process of data acquisition being limited to only those cases where surgery was performed, and tissue blocks were retrieved for biobanking rather than a comprehensive recording of all breast cancer cases diagnosed.

One of the problems in this collection was that the number of years for which the data was available and the year from which the data was available was not the same across the five centers. This may not matter much in terms of epidemiology data but treatment regimens have undergone changes through the years, and this may cause a bias in our analysis of survival data. This lacuna may be filled in future by prospective data collection.

Table 3: Number of cases where disease recurrence and mortality was recorded by the time in years postsurgery

Years after Surgery	Number of cases	Recurrence	Death due to disease
<1 year	7	6	3
1 to 2 years	6	2	5
2 to 3 years	9	0	7
3 to 4 years	18	2	3
4 to 5 years	26	2	3
>5 years	36	0	2

Following the guidelines for the treatment options, our study highlighted some of the significant differences in the choices of surgery and chemotherapy options at each center. Data collected from each of the participating physicians showed that the preference of the surgeon or the medical oncologist played a role in determining the type of surgery or the choice of chemotherapy regimen. However, this bias may be overcome if a larger data set that included all the cancer cases from each center was available. This observation was proven correct as our results showed a good correlation between the type of surgery used and the stage of TNBC when data from all the centers were pooled for analysis.

In our study, in 41% of Stage 1 cases, BCS was the choice of surgery, whereas it was about 31% in Stage 2a cases. MRM was the choice of surgery in cases that were Stage 2b or higher wherein more than 79% of the cases underwent mastectomy.

A combination of surgery and chemotherapy is recommended by the NCCN guidelines for the treatment

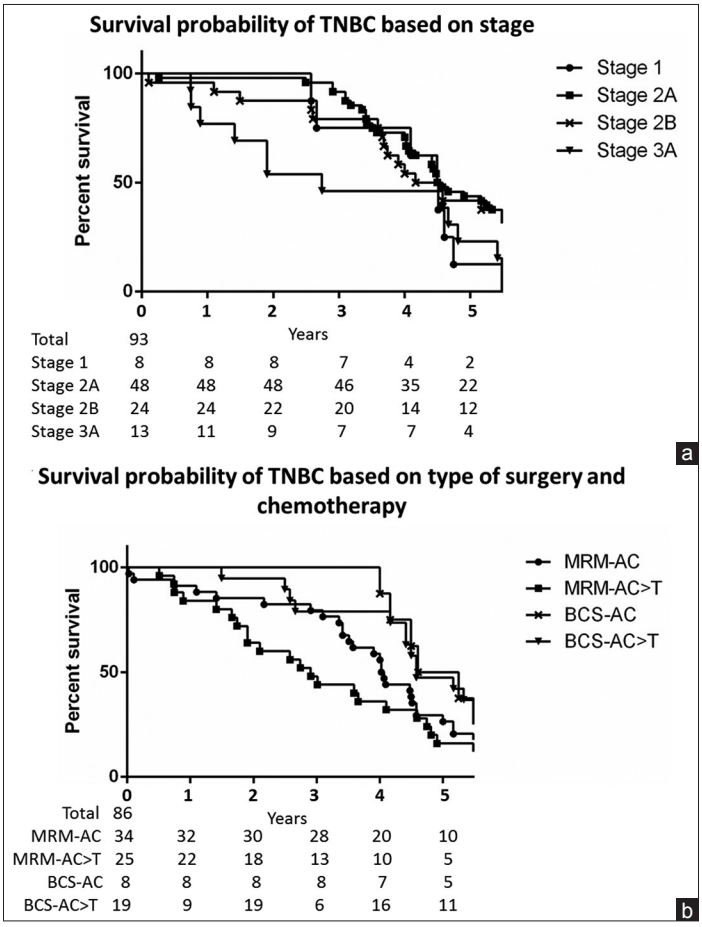

Figure 5: (a) Kaplan–Meier analysis of the survival probability based on the type of surgery and the chemotherapy administered. The total number of cases per group is depicted at the far left and was the starting number. The number of survivors at the end of each year was mentioned under each time point. (b) Percent survival of triple-negative breast cancer cases per year based on the stage of cancer depicted by Kaplan–Meier survival curves. The number of cases per stage is shown at the far left and is the starting numbers for the analysis. The number of survivors at the end of every year is mentioned under each time point

of breast cancer. In the case of TNBC, only standard chemotherapy is used, but generally the choice of the chemotherapy is guided by many factors which may not only be dependent on the stage or the aggressiveness of the disease but also on the age and the general health of the patient.

One variable in our study was that the number of years for which the data was available and the year from which the data was available was not the same across the five centers. This may not matter much in terms of epidemiology data but treatment regimens have undergone changes through the years, and this may cause a bias in our analysis of survival data.

With advances in the understanding of breast cancer disease biology, the therapeutic regimen has also undergone a progressive change. Rather than using a single class of chemotherapy agent, a combination of two or more agents that belong to multiple classes have been shown to provide better outcomes in multiple clinical trials.[14] Our data suggest that ACs alone were mainly used for cases confined to Stages 1 and 2a. For all stages higher than 2a, the choice was a combination of both ACs and taxanes. Taxanes by themselves were rarely used. Data from this study also show that the use of chemotherapy in the neoadjuvant setting was not common, with information available for only 25 cases of NACT usage out of 257. Whenever neoadjuvant was used, either ACs alone or in combination with taxanes were used in equal measure.

Posttreatment follow-up data for 5 years were limited to 102 cases out of 257. The maximal follow-up was more than 5 years, but most of the cases from Stage 3b and above were lost to follow-up. Due to lack of follow-up data for many cases beyond 3 years, the survival analysis presented in this study was based on the last follow-up date rather than the known death or known recurrence, hence actual OS is likely to be better than what is shown here.

Previous data from both International and Indian groups have shown that the median survival was 4.5 years in TNBC compared to 6 years in other cancers.[7,13] Data from our study show that irrespective of the treatment procedure, the 3-year survival was more than 85%, which was slightly higher than the 80% reported by Suresh *et al*.,[13] If broken down according to the type of surgery and chemotherapy, cases wherein BCS was the choice of surgery had a higher 3-year OS. In this group, the AC alone arm had a 100% OS for 3 years, whereas the ACs and taxanes combination arm had 79% OS. However, the ACs alone arm had only 8 cases compared to 19 cases in the combination arm and may not be representative.

A similar trend was observed in the MRM group. Cases where ACs were used alone had 79% 3-year OS compared to only 48% in the combination arm. It would be interesting to study the possible effects of higher potency of the combination chemotherapy in the decreased OS of this group. However, with the limited data, definitive conclusion on this was not possible. It should be noted that the effect of choice of surgery on OS may be limited to Stage 1 and 2a due to their being generally nonaggressive. The higher 3 year survival rate in cases from Stages 1, 2a, and 2b in our study also supports the overall treatment choices.

Conclusions

The results presented in this study are rather encouraging pertaining to the prognosis and treatment of TNBC in India. The occurrence rates were similar to that reported in some Western studies, and the median age of diagnosis was no different from other breast cancer cases. The 3-year prognosis and survival of the TNBC patients were on par with global average.

Collection of data pertaining to breast cancer cases is an ongoing process for us, and more data are being obtained continuously. The data and the samples being collected as part of the biobanking process opens up more avenues to understand novel aspects of TNBC and may allow for a deeper understanding of higher incidence in some areas, potential causes of treatment failure, prevalence of BRCA1/2 mutations, and family history. Studies on the role of cancer stem cells, tumor-infiltrating lymphocytes, to name a few would not only be useful in understanding the biology of the disease but also in finding newer and better treatment options. Our collection of both the data and the samples would go a long way in such studies.

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Conflicts of interest

There are no conflicts of interest.

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