Usefulness of three protein signatures (Mastocheck) for follow-up after breast cancer surgery

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Original Article

Usefulness of three protein signatures (Mastocheck) for follow-up after breast cancer surgery

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Abstract

Purpose: This study aimed to evaluate whether the three protein markers that we had developed previously for early breast cancer diagnosis, are useful for postoperative follow-up.

Methods: Of 111 patients with breast cancer that were prospectively enrolled and underwent blood tests before, 8-weeks, 6-months, or 1-year after surgery, 53 underwent serial tests preoperatively to 1-year postoperatively. Protein biomarker changes were analyzed before and after surgery. Moreover, 145 patients who underwent surgery >1 year previously, were prospectively enrolled into the recurrence and non-recurrence (no evidence of disease; NED) groups. Using traditional and artificial intelligence analysis methods, we determined whether the biomarkers could identify disease presence or absence, and evaluated whether significant correlation occurred in biomarker changes.

Results: Of the 111 included patients, 105 and 53 were followed-up for 8 weeks and 6 months to 1 year after surgery, respectively. Preoperatively, biomarker diagnostic sensitivity was 73.0%; 8-weeks and 6-months to 1-year after surgery, normalized blood biomarker level occurred in 67.6% and 86.8% of patients, respectively. Among 53 patients with serial tests, preoperative test sensitivity was 73.6%, which normalized after 6 month in 86.8%. Greater than 1-year after surgery, 38/63 patients with recurrence developed cancer, and 66/81 NED patients were cancer-free (62.5% sensitivity; 81.5% specificity). The upgraded version of biomarker analysis confirmed a further improved performance (with recurrence, 82.5% sensitivity; with NED, 87.7% specificity).

Conclusions: Blood protein signatures (Mastocheck) developed for early breast cancer diagnosis normalized over time postoperatively, and could be used as a biological monitoring test after breast cancer surgery.

Keywords: Breast neoplasms, Blood proteins, Proteomics, Biologic monitoring

Abbreviations:

AI, artificial intelligence; APOC1, apolipoprotein C-1; CAH1, carbonic anhydride1; IRB, Institutional Review Board; LC-MS/MS, liquid chromatography–mass spectrometry; MRM, multiple reaction monitoring; NCHL1, neural cell adhesion molecule L1-like protein;

Introduction

Breast cancer is the most commonly diagnosed cancer in women (24.2%, i.e. about one in four new cancer cases worldwide), and of 185 countries reported in GLOBOCAN 2018, breast cancer was the most common in 154 [1]. Breast cancer is also the second leading cause of cancer deaths in women (after lung cancer) [2]. Therefore, early diagnosis and proper follow-up are important. Traditionally, diagnosis and follow-up of breast cancer cases is based on physical examination and imaging using mammography. However, Asian women (including Korean and young women), have high rates of dense breast, low mammography sensitivity, and high false positive rates, which limit examination and follow-up observations [3, 4]. Mammography also causes severe pain during testing, and in young women, harm from irradiation may outweigh the benefits [5]. There is also the challenge of repetitive tests due to differences in performance levels or functionality of old machines. Therefore, recently, several ultrasound examinations are conducted; however, additional costs are incurred and the results may still vary due to differences in investigators level of proficiency [6]. Therefore, more objective and accurate diagnosis and tracking methods are needed.

We have recently developed and reported a new diagnostic technology called “Mastocheck,” for which the sensitivity, specificity, and accuracy of 71.6%, 85.3%, and 77%, respectively, were confirmed in a large-scale retrospective study [7]. Mastocheck, a new technology that is effective in early breast cancer diagnosis, works by quantifying and analyzing these three proteins in the blood: carbonic anhydride1 (CAH1), neural cell adhesion molecule L1-like protein (NCHL1), and apolipoprotein C-1 (APOC1) using multiple reaction monitoring (MRM)-based proteomics techniques [7, 8]. In a previous study, better performance was confirmed compared to that of the existing technology, mammography [9].

The purpose of this study was to evaluate whether the three protein signatures developed for early breast cancer diagnosis in previous studies are useful for postoperative follow-up. We also aimed to improve the power of accuracy of Mastocheck by adding eight markers to the previously developed three markers. Artificial intelligence (AI) analysis was also performed to improve the usefulness of the upgraded version of biomarkers for diagnosis and follow-up observation.

Methods

For patients that were diagnosed with breast cancer, blood was collected before surgery to determine the baseline value, and follow-up tests were performed, 8 weeks and 6 months to 1 year after surgery. In patients who were undergoing outpatient follow-up greater than 1-year after surgery, blood samples were also collected according to the outpatient visit schedule, to determine whether Mastocheck could detect recurrence. Blood samples collected at all stages were analyzed using the three-protein signatures (Mastocheck) developed in previous studies. Similarly, an analysis was conducted using a new algorithm in an upgraded version of Mastocheck, including eight additional candidate proteins.

Patients and study design

Comparison of Mastocheck, preoperatively and postoperatively, in patients with breast cancer

Among the patients who underwent surgery at Seoul National University Hospital for breast cancer from August 2018 to December 2020, 153 consenting patients were prospectively enrolled. After excluding 42 patients who withdrew consent during the follow-up period, 111 patients, with 111 preoperative blood samples and 105 samples at 8 weeks outpatient visit after surgery were included. Overall, 53 patients were followed-up throughout, from preoperative, to 8 weeks and 6 month postoperative.

The blood samples collected were sent to the laboratory, stored in a deep freezer below -60°C, and quantified using a mass spectrometer. The same researcher pre-processed and repeated the experiments two to three times to control the quality of the blood samples. The results of the experiments were obtained by using a breast cancer diagnosis algorithm developed in a previous study. Blood analysis was performed at regular intervals before and after surgery to determine the quantitative changes in the levels of individual proteins in the blood, according to cancer status in the body. Some of the collected blood samples were used to determine the performance of the new biomarkers in the upgraded version of Mastocheck currently under development.

Ethics approval

This study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (Approval No. D-1905-175-1036), and the study complied with the principles of the Declaration of Helsinki. The research design is shown in Fig 1. Table 1 shows the list of candidate proteins added to the newly-developed biomarkers.

Quantitative protein analysis

Quantitative analysis of the three proteins was performed using commercially available software (Analyst version 1.6, AB SCIEX, Framingham, USA) and reagent solutions (dithioerythritol [DTT], iodoacetamide [IAA], urea, and trypsin). A mass spectrometer (API 5000, AB Sciex, USA [Medical Device License No. Seoul, Korea 10-1245]) was used to perform liquid chromatography–mass spectrometry (LC-MS/MS) in MRM mode [7].

Statistical analysis

First, we identified whether there were significant differences in included variables between the two groups diagnosed with and without cancer, according to the Mastocheck results. Second, changes in Mastocheck values before and after surgery in patients with breast cancer were evaluated, and whether having cancer affected the marker values was examined to determine whether there was a correlation with the cancer status over time. Clinico-pathological information of patients with breast cancer was collected from the electronic medical records, to determine variables associated with biomarker changes before and longer than one year after surgery, and in patients who were diagnosed with and without recurrence during follow-up observation. For statistical analysis, IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9 were used.

Result

Comparison of Mastocheck, preoperatively and postoperatively, in patients with breast cancer

Overall, 111 patients were tested preoperatively, and the sensitivity of 73.0% was superior to that (71.6%) identified in the previous study. The clinical pathological characteristics of these patients are shown in Table 2.

Blood samples were collected before surgery, and about 4 (116 days) and 13 months (399 days) after surgery. Of the 105 patients with 8 weeks postoperative blood samples, 71 had normalized blood biomarker level (67.6%) while for 34 patients, the levels were still in the range of cancer. Of the 53 patients with all the three time-period blood samples, 47 (88.7%) had normalized blood biomarker level after the 1-year period. The overall tendency is shown in Fig 2.

The consecutive analysis of data obtained before surgery to the third blood collection included the 53 patients. As with the overall tendency, Mastocheck was normalized over time after surgery, and the changes in Mastocheck and individual markers are shown in Fig 3. Previous studies of each individual marker show that in breast cancer, CA1 and NCHL1 levels increased while that of APOC1 decreased [10-12]. In individual markers before and after surgery, CA1 and NCHL1 levels decreased after surgery, and APOC1 tended to increase after surgery; all changes in individual markers were statistically significant (*p-value*; APOC1=0.0054, CA1<0.001, NCHL1 = 0.0244). Mastocheck showed a statistically significant decreasing tendency postoperatively, compared to preoperatively ($p \leq 0.001$).

The characteristics of individual marker changes included that APOC1 tended to increase after surgery compared to before surgery and then decreased slightly over time. However, it also maintained a higher value than the preoperative value at greater than 1-year after surgery. CA1 increased slightly after surgery and then decreased over time, while CHL1 tended to decrease continuously after surgery. The Mastocheck value decreased sharply after surgery and then slightly increased over time, but still remained lower than the cut-off value. It is believed that the influence of APOC1 played a greater role in the algorithm, showing a similar level of change in Mastocheck. The analysis results obtained by substituting Mastocheck with a new algorithm, the upgraded version of Mastocheck, showed the before surgery sensitivity of 78.7%, and the normalized value of 76.5% at greater than 1-year after surgery. These results were based on a small sample size; therefore, it was difficult to determine the final performance. However, the finding is considered a positive result for the development of new markers.

Comparison between patients with and without recurrence among those followed-up for greater than 1-year after the standard treatment

Patients visiting the outpatient clinics at least one year after surgery were randomly enrolled, and the difference in Mastocheck between the two groups with and without recurrence was determined. Of 144 patients enrolled, 63 and 81 were with and without recurrence, respectively. Of the 63 with recurrence, 38 (60.3%) were diagnosed with cancer based on Mastocheck while 65 of 81 (80.2%) without recurrence were diagnosed as cancer-free; indicating an accuracy of 71.5% in the followed-up patients. According to the upgraded version of AI analysis including eight additional protein markers, 52 of 63 (82.5%) patients with recurrence were diagnosed with cancer while 71 of 81 (87.7%) without recurrence were diagnosed as cancer-free, indicating an improved accuracy to 85.4%. In the analysis of individual marker changes and Mastocheck, as shown in Fig 4, all markers tended to

increase in the recurrence group. Conversely, all markers were reduced in the group without recurrence. Except for APOC1, there were statistically significant differences between the two groups in individual markers and Mastocheck (*p-value*; APOC1=0.0713, CA1=0.0138, CHL1<0.001, and Mastocheck<0.001)

Discussion

Currently, screening tests for diagnosing breast cancer are limited to imaging tests such as mammography and ultrasound. With mammography, the diagnostic sensitivity in dense breasts such as for young and Asian women is very low. Furthermore, tests that can be used universally are limited due to concerns about harm such as pain and radiation exposure. With breast ultrasound, now used to replace mammography, wide variations occur in the results according to the physician, and although the sensitivity is high, false positive is also high, which often leads to unnecessary biopsy. The initial blood test was developed to overcome such high variations in diagnoses as well as the limitations in adaptation to be tested, and to ensure the convenience and reproducibility of the test results.

Mastocheck has a reasonable level of performance for early breast cancer diagnosis in previous studies [7]. Patients diagnosed with breast cancer by the currently used test method in the clinical field will undergo surgery after neoadjuvant chemotherapy depending on the stage. Follow-up after surgery also relies on imaging tests such as mammography and ultrasound. CA15-3 blood test, has a very low sensitivity, so it is difficult to expect its role as a tumor marker. In fact, only one out of the 111 patients enrolled in this study had a high preoperative CA15-3 value, while the rest all had normal levels. In addition, postoperative mammography can cause more pain than before surgery, and there are limitations to follow-up tests due to the distortion of the breast parenchymal gland caused by surgery. We are continuously conducting research to develop an objective, reproducible, and universally applicable test.

The main purpose of this study was to determine whether Mastocheck, a blood test developed for breast cancer diagnosis, is valid, through postoperative follow-up examinations. Thereby, the performance of Mastocheck through prospective blood collection, showed a sensitivity of 73%, similar to those of previous studies. Before and after surgery, follow-up tests were performed over a certain period to determine changes in protein levels in the blood and whether the changes could reflect the cancer condition over a continuous period of time.

In the previous study, a new diagnostic marker was developed for early diagnosis of breast cancer using 1,129 stored blood samples, analyzed through quantification and optimization processes. In addition, correlation evaluation with other cancers confirmed that it to be a unique marker for breast cancer diagnosis. Various methods such as correlation evaluation with anesthesia were conducted to develop an objective and universal diagnostic marker for breast cancer diagnosis [7]. In addition, the sensitivity (59.2% -> 93.0%) and accuracy (69.0% -> 85.4%) of Mastocheck in dense breast compared to the single mammography when combined, were evaluated [9]. A study comparing ultrasound alone and in combination also identified reduced false positive rates of ultrasound, and unnecessary biopsy was avoided by improving the diagnostic specificity [13].

The success of breast cancer treatment is ultimately defined by clinical endpoints such as survival. However, measuring these clinical endpoints requires long-term follow-up, and it is difficult to predict the treatment of individual patients early in the course of treatment. Therefore, it is important to have biomarkers that can predict the most effective treatment or measure early response to therapy in the course of treatment. The treatment of breast cancer has been guided for many years by tissue-based biomarkers including estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) status [14, 15]. However, these traditional biomarkers have some limitations, such as the inability to assess the heterogeneity in breast cancer presentation [16, 17]. In addition, many studies have shown that CA 15-3, which is now widely used as a breast cancer-specific biomarker, are not available for early diagnosis as a single marker due to its low sensitivity and specificity, and because it showed no significant correlation with cancer metastasis during follow-up [18, 19]. Therefore, studies are ongoing to develop objective biomarkers in the absence of significant markers of the diagnosis and follow-up of breast cancer. The values of developed biomarkers are being evaluated and extensive clinical applications are being verified; however, limitations remain. Importantly, tests on receptor expression and gene profiling are generally conducted on individual tissue samples obtained during biopsy or surgery, making it difficult to use them for screening purposes. Therefore, there is still the need for a broader, more objective, and simpler test method.

Proteomics can actually determine the level of protein in the cell, which helps explain where they are located, and which other proteins interact. The effects of cell environment can also be observed. In other words, it allows us to see how the levels of proteins change and how cells react [20]. For this reason, the researchers developed biomarkers using proteomics techniques. Mastocheck is the result of years of experimentation with various designs to confirm the usefulness of the method for breast cancer diagnosis. Many experiments conducted on the development till date, have focused on diagnosis.

Our data suggest the possibility of Mastocheck as a postoperative follow-up method, which is normalized in >86% of patients after 1-year of surgery. The limitations of this study are that it is an ongoing study, and it was difficult to generalize the interpretation to all the patients with breast cancer due to the low number of subjects analyzed so far, and the short duration of follow-up observations. It is also likely that discussions on the appropriate timing of follow-up examinations after surgery will be needed. Despite these limitations, it is meaningful that such studies can be proposed in the future, because analysis including multi markers was conducted to identify blood-based markers that can be monitored after treatment. The ongoing research is both a limitation and a strength at the same time. More data will be accumulated and changes in markers before and after chemotherapy as well as before and after surgery are being studied. The method could provide a new paradigm for developing biomarkers that are useful for follow-up observations in the future.

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Declarations

Conflict of Interest Yumi Kim and Hong-Kyu Kim own unlisted stock in Bertis Inc. Sungsoo Kim and Kyung-guen Ahn employed in Bertis Inc. Dong-Young Noh is Co-CEO of Bertis Inc. Changjin Lim declare has no

conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent This prospective study was approved by the institutional review board, and the informed consent requirement was waived.

References

1. Bray F, et al. (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424. https://doi.org/10.3322/caac.21492

2. Azamjah N, Soltan-Zadeh Y, Zayeri F (2019) Global trend of breast cancer mortality rate: A 25-year study. Asian Pac J Cancer Prev 20(7):2015–2020. https://doi.org/10.31557/APJCP.2019.20.7.2015

3. Kim YJ, et al. (2017) Analysis of participant factors that affect the diagnostic performance of screening mammography: A report of the Alliance for Breast Cancer Screening in Korea. Korean J Radiol 18(4):624–631. https://doi.org/10.3348/kjr.2017.18.4.624

4. Rafferty EA, et al. (2016) Breast cancer screening using Tomosynthesis and digital mammography in dense and nondense breasts. JAMA 315(16):1784–1786. https://doi.org/10.1001/jama.2016.1708

5. Myers ER, et al. (2015) Benefits and harms of breast cancer screening: A systematic review. JAMA 314(15):1615–1634. https://doi.org/10.1001/jama.2015.13183

6. Wojcinski S, et al. (2011) Optimizing breast cancer follow-up: Diagnostic value and costs of additional routine breast ultrasound. Ultrasound Med Biol 37(2):198–206. https://doi.org/10.1016/j.ultrasmedbio.2010.10.024

7. Kim Y, et al. (2019) A validation study of a multiple reaction monitoring-based proteomic assay to diagnose breast cancer. J Breast Cancer 22(4):579–586. https://doi.org/10.4048/jbc.2019.22.e57

8. Lee HB, et al. (2015) Development and validation of a novel plasma protein signature for breast cancer diagnosis by using multiple reaction monitoring-based mass spectrometry. Anticancer Res 35(11):6271–6279

9. Kim Y, et al. (2019) Efficacy of Mastocheck for screening of Early breast cancer: Comparison with screening mammography. J Breast Dis 7(2):59–64. https://doi.org/10.14449/jbd.2019.7.2.59

10. Li J et al. (2019) New power of self-assembling carbonic anhydrase inhibitor: Short peptide–constructed nanofibers inspire hypoxic cancer therapy. Sci Adv 5(9):eaax0937. https://doi.org/10.1126/sciadv.aax0937

11. He LH, et al. (2013) CHL1 is involved in human breast tumorigenesis and progression. Biochem Biophys Res Commun 438(2):433–438. https://doi.org/10.1016/j.bbrc.2013.07.093

12. Sun Y, et al. (2016) Identification of apolipoprotein *C*-I peptides as a potential biomarker and its biological roles in breast cancer. Med Sci Monit 22:1152–1160. https://doi.org/10.12659/msm.896531

13. Ha SM, et al (2022) Diagnostic performance improvement with combined use of proteomics biomarker assay and breast ultrasound. Breast Cancer Res Treat 192(3):541–552. https://doi.org/10.1007/s10549-022-06527-1

14. Harris L, et al. (2007) American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 25(33):5287–5312. https://doi.org/10.1200/JCO.2007.14.2364

15. Patani N, Martin LA, Dowsett M (2013) Biomarkers for the clinical management of breast cancer: International perspective. Int J Cancer 133(1):1–13. https://doi.org/10.1002/ijc.27997

16. Amir E, et al. (2012) Prospective study evaluating the impact of tissue confirmation of metastatic disease in patients with breast cancer. J Clin Oncol 30(6):587–592. https://doi.org/10.1200/JCO.2010.33.5232

17. Chang HJ, et al. (2011) Discordant human epidermal growth factor receptor 2 and hormone receptor status in primary and metastatic breast cancer and response to trastuzumab. Jpn J Clin Oncol 41(5):593–599. https://doi.org/10.1093/jjco/hyr020

18. Rasmy A et al. (2016) Correlation of preoperative Ki67 and serum CA15.3 levels with outcome in Early breast cancers - A multi institutional study. Asian Pac J Cancer Prev 17(7):3595–3600

19. Elfagieh M et al. (2012) Serum tumour markers as a diagnostic and prognostic tool in Libyan breast cancer. Tumour Biol 33(6):2371–2377. https://doi.org/10.1007/s13277-012-0500-6

20. Wilhelm M, et al. (2014) Mass-spectrometry-based draft of the human proteome. Nature 509(7502):582–587. https://doi.org/10.1038/nature13319

Figures

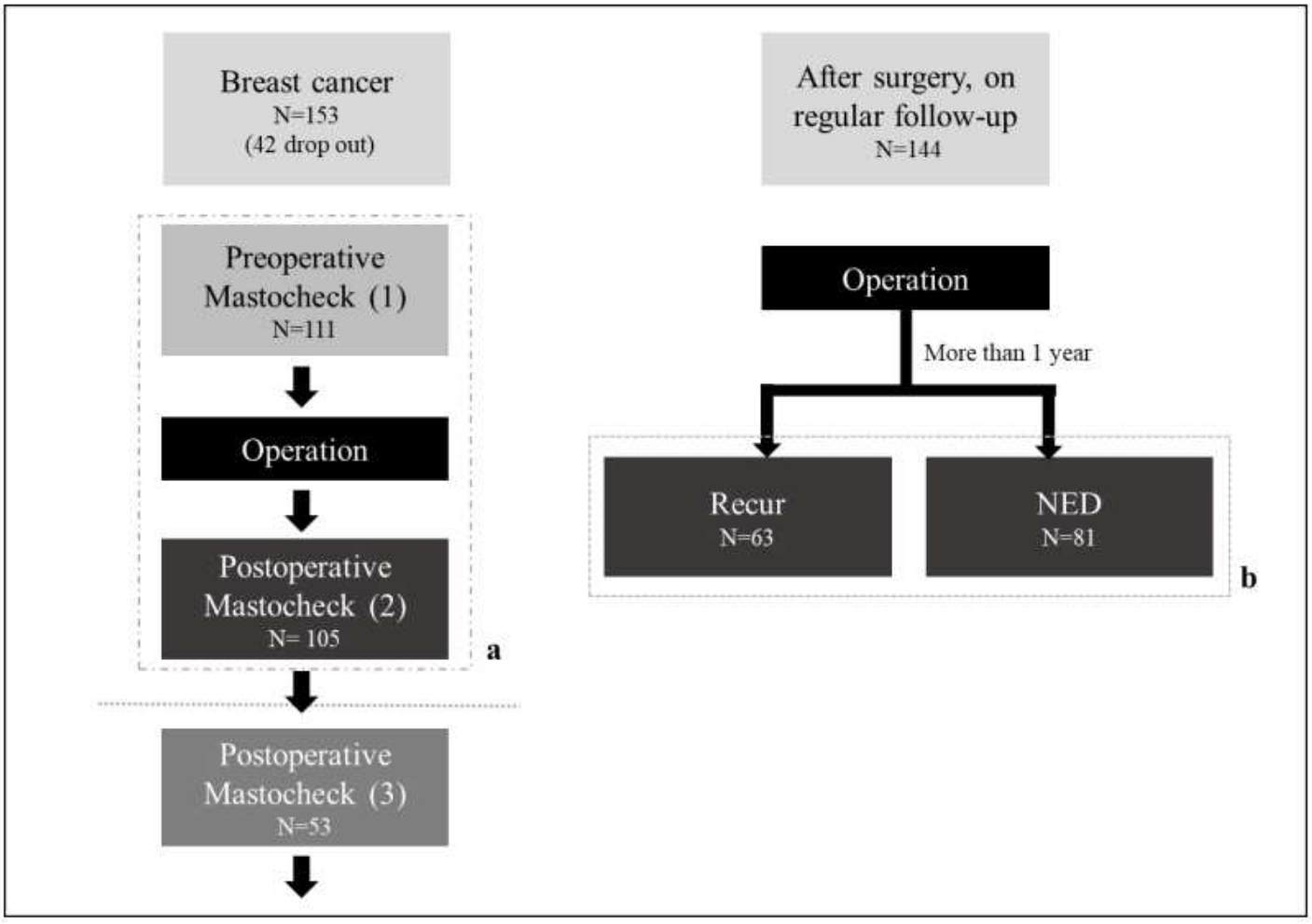

Figure 1

Study design schema

a. Comparison of preoperative and postoperative Mastocheck® values of breast cancer patients

b. Comparison of Mastocheck values in patients with recurrence and NED (No evidence of Disease)

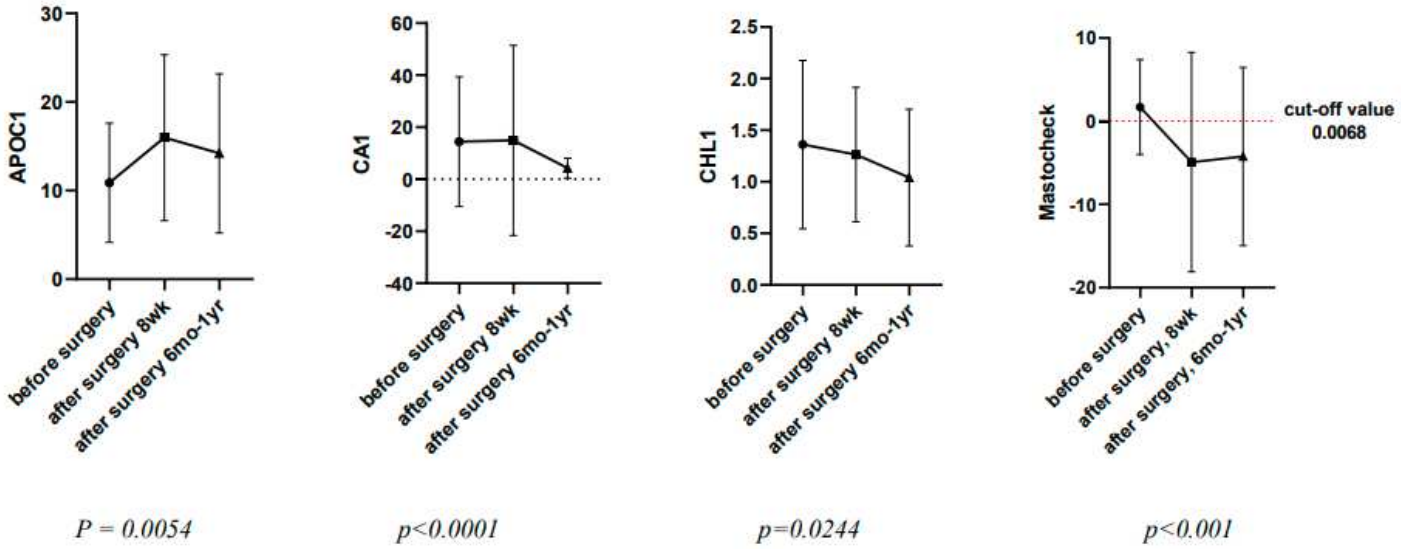

Figure 2

Changes in individual markers and Mastocheck before and after surgery (all samples)

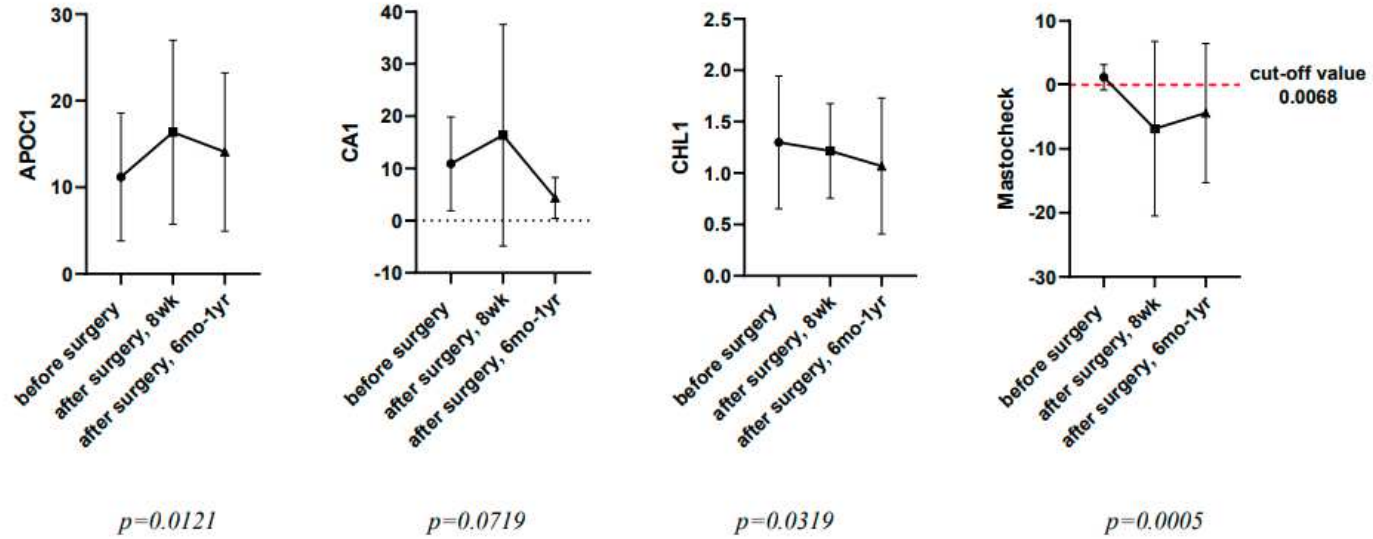

Figure 3

Results of continuous analysis for more than one year before and after surgery. (53 paired samples)

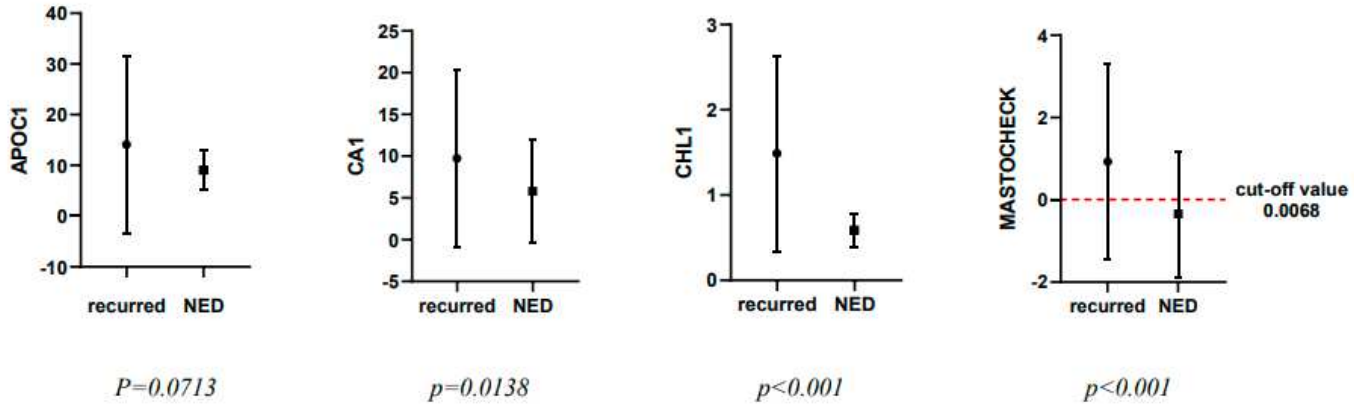

Figure 4

Comparison between patients with and without recurrence among those followed-up for more than 1-year after surgery

NED ; No Evidence of Disease

Supplementary Files

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- tableMCfuApr2022.pdf