Therapeutic importance of timely immunophenotyping of breast cancer in a resource-constrained setting: a retrospective hospital-based cohort study

Arturo López Pineda, PhD[1,§,*], Mario Felipe Rodríguez Moran, MD[2,§], Cleto Álvarez Aguilar, MD, MS[2,3], Sarah Marlenne Fuentes Valle, MD[3], Román Acosta Rosales, MD[3], Carlos D. Bustamante, PhD[1]

[1] Stanford University. Department of Biomedical Data Science. Stanford, California, USA

[2] Universidad Michoacana de San Nicolas de Hidalgo. Faculty of Medical and Biological Sciences "Dr. Ignacio Chavez". Morelia, Michoacan, Mexico.

[3] Mexican Institute of Social Security. Michoacan Delegation. Morelia, Michoacan, Mexico.

[§] Authors contributed equally to this work.

[*] Corresponding author

Address correspondence to:

Arturo Lopez Pineda, PhD

Department of Biomedical Data Science

Stanford University School of Medicine

365 Lasuen Street, Stanford, CA, 94305, USA

Email: arturolp@stanford.edu

Abstract

Background. The Mexican Consensus of 2015 on diagnosis and treatment of breast cancer proposes the use of surrogate classification based on immunohistochemistry (IHC). In this study, we assessed the therapeutic value of this consensus recommendation, in the absence of gene expression data, as a tool for providing opportune and precise care.

Methods and Findings. In this retrospective hospital-based cohort study, we reviewed medical records of all suspected breast cancer patients in 2014 treated at the General Regional Hospital No. 1, of the Mexican Institute of Social Security (IMSS), located in Charo, Michoacan (western Mexico). Then, we followed the medical history of those patients with IHC testing until March 2017. The trajectory of patients was recorded to discover common patterns, and turnaround time for IHC testing. There were 402 suspected breast cancer patients in 2014, of which 30 had been tested for some IHC biomarkers (ER, PR, HER2). The surrogate subtyping allowed doctors to adjust (56.7 %) or confirm (43.3 %) the initial therapeutic regimen. Opportune IHC testing was found to be beneficial when it was available before or during the first rounds of chemotherapy.

Conclusions. The use of opportune immunohistochemistry provides improved outcomes for breast cancer patients. This result has the potential to become a roadmap for the implementation of precision medicine in Mexico.

Keywords: Breast Neoplasms; Immunohistochemistry; Tumor Biomarkers; Medical Records; Clinical Coding; Mexico

Abbreviations: IMSS, Mexican Institute of Social Security; HGR1, General Regional Hospital No. 1 in Charo, Michoacan, Mexico.

46 # 1. Introduction

47 Molecular subtyping for breast cancer tumors based on gene expression patterns have
48 been previously recognized to have prognostic value in the management and treatment of breast
49 cancer patients [1]. During the last decade, there have been several commercially accepted multi-
50 gene prognostic tests. Genomic prognostic tests have been associated with decreased adjuvant
51 chemotherapy use among patients, and better outcomes with cost-effective treatments [2].
52 However, the availability and expense of these genomic tests remain largely prohibitive for
53 constrain-resourced hospital settings, regardless of country income. For example, in the United
54 States, the use of the 21-gene recurrence score Oncotype DX has increased significantly over the
55 last decade due to insurance coverage, but it is estimated that only a quarter of eligible patients
56 have been tested [3].

57 In resource-constrained settings, the use of surrogate immunohistochemistry (IHC)-based
58 breast cancer subtypes presents as an alternative to molecular subtyping when there is no access
59 to gene expression data or high-throughput sequencing data. Despite the lowering costs of
60 genomic tests, these technologies are only available to researchers in select locations in the
61 world, and they are inaccessible to most patients worldwide. Alternatively, a panel of three IHC
62 markers—estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth
63 factor receptor 2 (HER2)—demonstrated its predictive value for chemotherapy response in
64 breast cancer [4]. There are other IHC markers that could be included for the prediction of
65 treatment response and prognosis; however, further studies are needed to select an optimized
66 surrogate panel [5].

67 The 2015 Mexican Consensus on diagnosis and treatment of breast cancer [6] is the main
68 working document for oncologists in the country. It recommends routine use of a 3-marker panel

69 (ER, PR, HER2) IHC approximation to identify four intrinsic breast cancer phenotypes (luminal
70 A, luminal B, HER-2, and triple negative). In the Mexican population, the estimated frequency
71 of ER/PR positive is 60%, HER2 positive 20.4%, and triple negative is 23.1% [7,8]. There is a
72 troubling onset of disease among younger breast cancer patients in Mexico (aged <40), with a
73 high prevalence of triple-negative breast cancers [9]. Furthermore, there is an increased mortality
74 trend associated with breast cancer in the country [10].

75 The Mexican clinical practice guidelines for screening, management, and treatment of
76 breast cancer [11-13] also recommends the use of IHC testing as part of the histopathology
77 studies. Despite these recommendations, the widespread use of genetic testing in Mexico remains
78 elusive due to constrained resources. However, IHC testing is widely available through hospital-
79 owned pathology laboratories, as well as through outsourced commercial laboratories across the
80 country.

81 In this study, we aimed to assess the timeliness of surrogate IHC biomarker testing in the
82 context of a resource-constrained hospital setting. Evaluating the therapeutic value for patients is
83 of critical importance, since the demand for more IHC testing will create new logistical
84 challenges in the healthcare system. The impact of acceptable or critical time delays is ultimately
85 not well defined, and it would be of great importance for hospital administrators to have a
86 reference of the value of timely IHC testing. As precision medicine continues to provide better
87 prognostic biomarkers, translating these benefits into clinical practice increasingly requires a
88 time-efficient approach from the entire healthcare system.

89

2. Methods

Ethical approval

This research was reviewed and approved by the Mexican Institute of Social Security (IMSS)'s National Scientific Research Committee and its National Bioethics Committee, under protocol number R-2017-785-010, and locally by IMSS' Local Research and Ethics Committee, under specific protocol number R-2017-1602-14 HGR1 Charo, Michoacan. Stanford's Institutional Review Board provided a non-human subject determination under eProtocol: 40704. Researchers only received access to non-identifiable data under the rigorous considerations of the applicable Mexican bylaws on health research, patient privacy, and electronic medical records. Consent was not required.

Hospital setting

The Mexican Institute of Social Security is a hybrid single-payer system with an integrated network of hospitals nationwide. In Michoacan (a state located in western Mexico), the General Regional Hospital No. 1 (HGR1) is the designated secondary-care facility for the IMSS-insured population, estimated at 1,288,695 people (28% of the state's population), out of 4,584,471 according to the 2015 census [14].

This hospital is in Charo, a suburban community adjacent to the Michoacan capital (Morelia), and it was built in 2012 to replace the old general hospital. HGR1 is the reference secondary-care facility, which serves as reference for seven General Zone Hospitals and 45 Family Medicine Units within the state of Michoacan. HGR1 has an oncology unit with a full complement of fixed staff and facilities available to all patients. It also offers pathology services, diagnostic imaging, and therapeutic capabilities with access to all approved drugs. Patients with

113 breast cancer who require chemotherapy are sent to the outpatient medical unit, and patients with
114 breast cancer who require radiation therapy are provided the service through an outsourced
115 private service in the same city. The personnel include gynecologists, medical oncologist,
116 surgical oncologists with significant breast cancer training, adequate numbers of nursing and
117 pharmacy staff, surgeons with significant training, and cancer pathologists. A self-assessment of
118 the services and facilities provided by these hospitals is S1 Text, which is based upon a modified
119 version of the oncological service self-assessment levels for low- and middle-income countries
120 developed by The International Society of Pediatric Oncology (SIOP) committee on Pediatric
121 Oncology in Developing Countries (PODC) [15].
122

123 **Patients**

124 The study population for this research was all cumulative breast cancer patients seen in
125 2014 by medical staff at the IMSS's General Regional Hospital No 1 in Charo, Michoacan. The
126 institution's breast cancer census was used to select patients that were seen in 2014 at HGR1. To
127 avoid selection bias in our study, we did not restrict the selection of patients to members of the
128 female sex or any age group. The inclusion criteria can be seen in Figure 1.

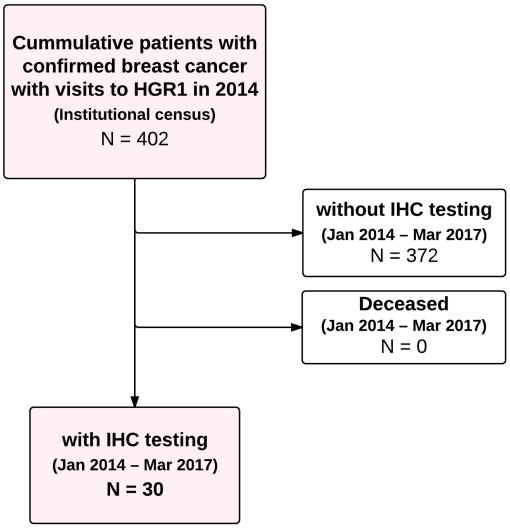


Figure 1. Inclusion criteria.

A patient with suspected breast cancer is referred from a primary-care facility to HGR1 after being examined by his/her family physicians. If the physician suspects the existence of a breast lesion, the patient undergoes an imaging assessment using the breast imaging-reporting and data system (BIRADS) score. Upon arriving at HGR1, the patient was either seen by the breast cancer clinic (BIRADS 0 and 3) for further imaging studies and/or fine-needle biopsy assessments, or directly referred to the medical oncology service (BIRADS 4, 5 and 6), at which point they would undergo surgical biopsies for pathology analysis. The gold-standard diagnosis of breast cancer would be provided by a board-certified pathologist, who would assign one of the C50 codes (malignant neoplasms of breast) from the international code of diseases version 10 (ICD10).

We reviewed the medical records (both electronic and paper) of all breast cancer patients at HGR1, and selected those whose biopsies had also been tested with IHC to detect ER, PR, HER2 antibodies; then, we followed the medical histories of those patients, ending with March

145 2017, making note of multiple medical visits to the breast cancer clinic, medical oncology
146 service, and/or surgical oncology service. From their medical records, we extracted information
147 about IHC testing (antibody ordered, date of ordering, date of results being obtained),
148 chemotherapy and hormonal drugs administered, and radiation sessions and surgical procedures
149 undertaken.
150

151 **Study design**

152 This was a retrospective hospital-based cohort study, using medical records collected
153 routinely as part of clinical care. The objective was to understand the therapeutic impact on
154 breast cancer patients of the time taken to test surrogate immunohistochemistry biomarkers (ER,
155 PR, HER2) in a resource-constrained hospital in western Mexico. We were interested in
156 reporting the frequency of treatment selection or treatment adjustment frequencies depending on
157 breast cancer subtyping and the turnaround times for surrogate immunohistochemistry biomarker
158 testing. The analysis included timeline trajectories and association rules. The STROBE checklist
159 is provided in S2 Text. All analyses were performed in R version 3.3.2.

160 *Timeline trajectories*. We explored the paper- and electronic-based medical records for
161 all patients with IHC testing, from the patient's first day at the hospital to the last follow-up
162 pertaining to this study (occurring before March 2017). First, author MFRM manually reviewed
163 the patient's medical records and curated a set of possible events (transactions) that occurred to
164 the patients of this study, annotating the time points for each event for each patient. Then, author
165 ALP annotated those events with standardized coding from the Unified Medical Language
166 System (UMLS). When disagreement occurred, both authors discussed the translation to assign a
167 UMLS code to the medical records in Spanish. Finally, similar events were grouped together in

168 fewer categories. The UMLS codes, UMLS descriptions, Spanish description, and groupings can

169 be seen in Table 1.

170

171 **Table 1**. List of medical events with UMLS codes and descriptions in English and Spanish.

UMLS code	UMLS description	Description in Spanish
Visit		
C0008952	Clinic Visits	*Atención inicial en la unidad de medicina familiar (primer nivel de atención).*
C2153644	Visit for: gynecological exam	*Atención inicial en la consulta de ginecología (segundo nivel de atención).*
Oncology		
C1620996	Oncology; primary focus of visit; work-up, evaluation, or staging at the time of cancer diagnosis or recurrence (for use in a medicare-approved demonstration project)	*Atención inicial en la consulta de oncología médica.*
C1617848	Oncology; primary focus of visit; expectant management of patient with evidence of cancer for whom no cancer-directed therapy is being administered or arranged at present; cancer-directed therapy might be considered in the future (for use in a medicare-approved demonstration project)	*Atención subsecuente en la consulta de oncología médica.*
Request lab		
C2186763	Request lab results from pathology	*Solicitud de estudios de patología.*
C2186756	Request lab results from hematology	*Solicitud de estudios básicos de laboratorio clínico.*
C2186777	Request lab results from x-ray	*Solicitud de estudios de imagen (tele de tórax, ultrasonido, mastografía).*
C2186774	Request lab results from CT	*Realización de estudios especiales de imagen (tomografía).*
C2186775	Request lab results from MRI	*Realización de estudios especiales de imagen (resonancia magnética).*
Biopsy / Pathology		
C0177666	Needle biopsy of breast	*Realización de biopsia con aguja fina o gruesa.*
C0585992	Surgical biopsy of breast	*Realización de biopsia por escisión quirúrgica.*
C0807321	Pathology report	*Reporte de estudio histopatológico.*
Chemotherapy		
C0086965	Selection for Treatment	*Selección de tipo de tratamiento (quimioterapia, radioterapia, cirugía).*
C4302504	Chemotherapy started	*Inicio de quimioterapia.*
Surgery / Radiation		
C0436382	Radiotherapy started	*Inicio de radioterapia.*

C0024881	Mastectomy	*Realización de mastectomía radical.*
C0851238	Lumpectomy of breast	*Realización de lumpectomía*
IHC results		
C3248285	Quantitative HER2 immunohistochemistry (IHC) evaluation of breast cancer consistent with the scoring system defined in the ASCO/CAP guidelines (PATH)	*Solicitud de estudios de inmunohistoquímica (Receptor de HER2).*
C3248286	Quantitative non-HER2 immunohistochemistry (IHC) evaluation of breast cancer (eg, testing for estrogen or progesterone receptors [ER/PR]) performed (PATH)	*Solicitud de estudios de inmunohistoquímica (Receptores de estrógeno y progesterona).*
Treatment adjusted		
C1627778	Treatment adjusted per protocol	*Ajuste de tratamiento de primera línea (posterior al primer ciclo de quimioterapia).*
C0419989	Hormone replacement therapy started	*Inicio de tratamiento de reemplazo hormonal.*
Hospitalization / Metastasis		
C0019993	Hospitalization	*Complicación de proceso oncológico (internamiento a causa de cáncer)*
C1458156	Recurrent Malignant Neoplasm	*Recaída de proceso oncológico (mismo sitio tumor primario).*
C3694291	Metastasis from malignant neoplasm of breast	*Reconocimiento de metástasis (tumor secundario).*
Remission		
C0687702	Cancer Remission	*Evolución favorable de paciente, mejoría clínica; remisión.*

Missing data. Missing data was assumed to be non-missing at random (NMAR), and the events from Table 1 were considered missing for a specific reason. For example, if the code C2186775 (request lab result from MRI) was missing from a patient's medical record, we left that value unassigned, since it was assumed that an MRI was not requested for that patient. There is a small chance that this assumption might be violated (and introduce some bias) if the physician did request the exam/procedure but forgot to write it down in the medical record. However, we did not impute any missing data, since that might be a larger source of bias for this EHR-based data.

Outliers. We assessed the time-to-event distribution for each event in Table 1, and reported the mean time using boxplots with whiskers. The outliers were identified using the 1.5

183 interquartile rule, but were not removed from the downstream analysis since undefined time-to-
184 event standards exist for these events.

185 *Clustering*. A heatmap with all the patients and events was generated. We used
186 hierarchical clustering to create dendograms. The distance matrix was generated with the time-
187 to-event matrix, filling all missing events with a negative number (–1000). Distance was
188 calculated with Pearson's correlation, while linkage was computed as complete.

189 *Association rules*. The Apriori algorithm [16] was used to discover association rules that
190 are common across multiple patients. Apriori first counts event occurrences to determine the
191 largest 1-item sets. Then, in subsequent steps, it iteratively tries to generate larger candidate item
192 sets, based on an incremental one-step from the previous step, and assign the strength of
193 evidence for that set of events based on the number of occurrences in the database. The Apriori
194 algorithm uses a bottom-up approach. The algorithm finalizes when no candidate list hast larger
195 item sets. The candidate generation step uses a breadth-first search to efficiently count candidate
196 item sets, and prune candidates with infrequent patterns. The algorithm does not consider the
197 time-to-event but only whether the event occurred and in what order it occurred for that patient.

198 **3. Results**

199 The epidemiological information obtained pertaining to this one IMSS hospital can be
200 seen in Table 2. The results show the patients with breast cancer in 2014 (N=402), which
201 correlate with national estimates for the country [10]. In this cohort, all patients were female. The
202 highest prevalence of cancer was within the 50-59 year old age group. The majority of patients
203 were diagnosed with early stages of cancer (stages I and II). However, only a few number of
204 patients (N=30) had undergone subtyping with IHC testing in accordance with the Mexican

205 Consensus. Even in this small cohort, we can still see the same higher prevalence of triple
206 negative breast cancer cases that was previously reported [17]. It is troubling to see that there
207 were 18 (4% of) patients younger than 40 years old.

208 At IMSS, patients with Breast Imaging Reporting and Data System (BIRADS) reports
209 scoring 1 or 2 are usually deemed low-risk for breast cancer and they have regular annual
210 screenings at their Family Medicine Unit (UMF). Meanwhile, patients with BIRADS reports 0
211 and 3 are sent to IMSS's secondary care breast cancer clinic for a closer screening (usually fine-
212 needle biopsy and 6-month screening); while patients with BIRADS reports 4, 5 or 6 are sent
213 directly to the secondary or tertiary care oncology services. At the IMSS-HGR1 hospital that we
214 investigated, 23 patients (6%) had a BIRADS score of 2 or lower, which were not supposed to be
215 sent to the secondary care facility. In these cases, patients had external biopsy investigations in
216 non-IMSS hospitals (usually private), and decided to continue their care for follow-up treatment
217 at the IMSS oncology services.

218

219 **Table 2**. Clinical characteristics of the cohort and receptor status for patients with IHC testing

Characteristic	Total (%)
Overall	**N = 402**
Sex	
Female	402
Male	0
Age at diagnosis	
<40	18 (4%)
40-49	62 (15%)
50-59	174 (43%)
60-69	127 (32%)
70+	21 (5%)
BIRADS	
0,1,2	23 (6%)
3	203 (50%)
4,5,6	162 (40%)
unknown	14 (3%)
Tumor stage	
I	85 (21%)

II	122 (30%)
III	84 (21%)
IV	19 (5%)
unknown	92 (23%)
Patients with IHC testing	**N = 30**
ER testing	
positive	17 (57%)
negative	11 (37%)
unknown	2 (7%)
PR testing	
positive	16 (53%)
negative	12 (40%)
unknown	2 (7%)
HER2 testing	
positive	5 (17%)
negative	23 (77%)
unknown	2 (7%)
Additional IHC testing	
Ki67, P53	1 (3%)
unknown	29 (97%)
Subtype according to the Consensus	14 (47%)
Luminal A	3 (10%)
Luminal B	9 (30%)
Basal-like / Triple negative	2 (7%)
HER2	2 (7%)
not enough information to assign	

Clustering analysis

A hierarchical clustering and heatmap of time-to-events is shown in Figure 2. The time in which each event occurred for each patient is measured using information found in their medical record (in days, positive). To be able to create a dissimilarity matrix to calculate distance between patients, all missing values were assigned a negative value of -1000. It is important to note that this figure does not provide a context for the ordering in which events occurred, but it can still help to provide information about patients with similar trajectories. Three main groups of patients (or clusters) were identified in this graph.

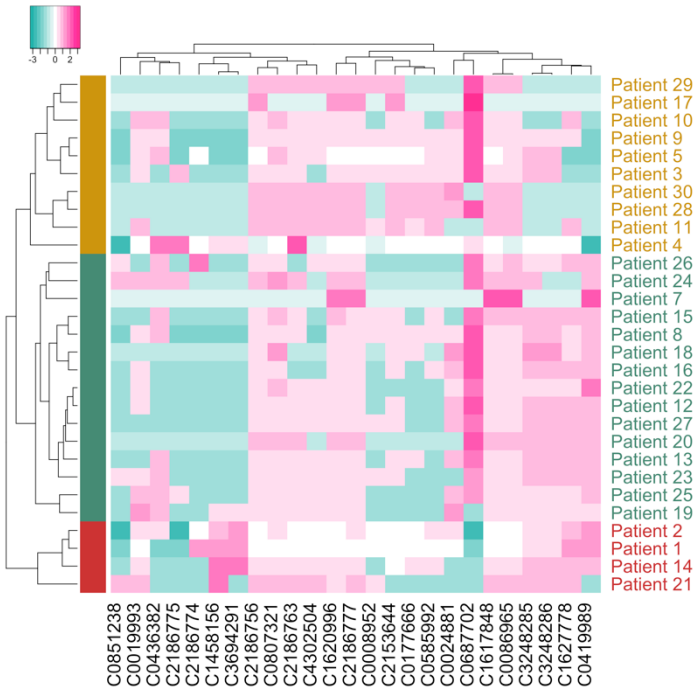


Figure 2. Heatmap of time-to-event with patient clustering.

Patient trajectories

Medical trajectories for patients in the cohort with IHC testing can be seen in Figure 3, clustered according to the groupings shown in Figure 2. Each patient is represented in a panel (rectangle) with colored bars, indicating the events that a patient experienced in the IMSS hospital. Each row represents one year of follow-up treatment for that patient, which can be from one to four rows (because the time maximum time span is three years and five months). The length of each colored bar represents the time between the occurrence of an event and the event that preceded it. Although events do not occur continuously, but, rather, happen at single time points (e.g. a patient visit, obtaining the results of an x-ray), the visual representation shown in Figure 3 provides a sense of how much time was required for each event to occur, assuming nothing else happened at the same time.

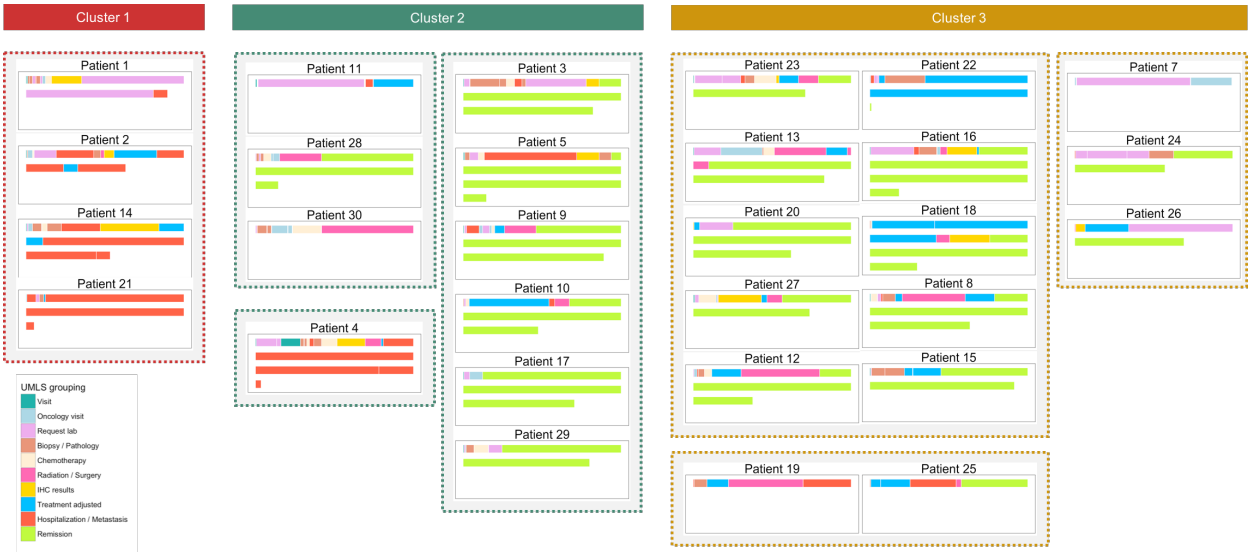


Figure 3. Medical trajectories of the cohort with IHC testing. Each patient is represented by a rectangle, with rows representing each year of follow-up treatment. Events are color-coded according to the type of event, and the length of the bar represents the duration between the occurrence of an event and the event that preceded it.

Turnaround time

The time-to-event for all events is shown in Figure 4. It is measured as the number of days that it took for an event to occur, from the initial visit to the hospital stay (day 0). From this Figure, it is easy to see that visiting the hospital or beginning treatment occurred relatively at the beginning of the patients' trajectories, while events that typically take place later did in fact occur at a posterior time, such as remission or metastasis. Also, one can see there is a wide distribution of time, when requesting laboratory/imaging testing, which coincides with the continuity of care.

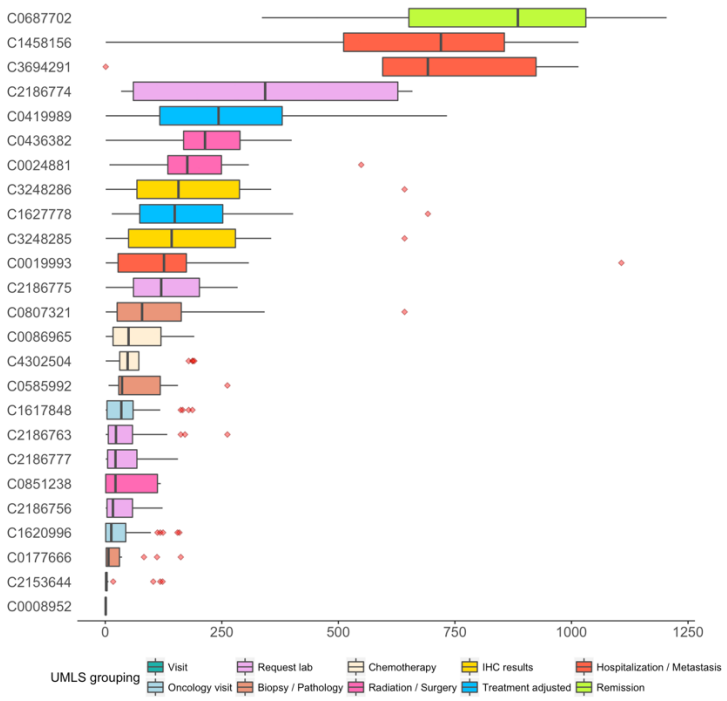


Figure 4. Boxplots for time-to-event

The turnaround time for immunohistochemistry (IHC) testing can be seen in Figure 5. Only 20 patients had information in their medical records relating to the timing of IHC testing. Although this service is referred to in the IMSS medical records, it is subcontracted to a private laboratory. There was large variation surrounding two key variables: a) the time needed to request IHC testing relative to the first day of follow-up treatment at the hospital, and b) the turnaround time to obtain those results. For the first one, the average time of request was 117 days (95% C.I. 80 – 154 days). For the second one, the average turnaround time for obtaining those results was 56 days (95% C.I. 36 – 77 days).

There were 15 out of 20 patients whose treatments were adjusted, in accordance with IMSS protocol, after obtaining the results of the IHC testing. Overall, for the 30 patients with

IHC testing, 17 patients (56.7%) had their treatments adjusted, and 13 patients (43.3%) had their treatments confirmed.

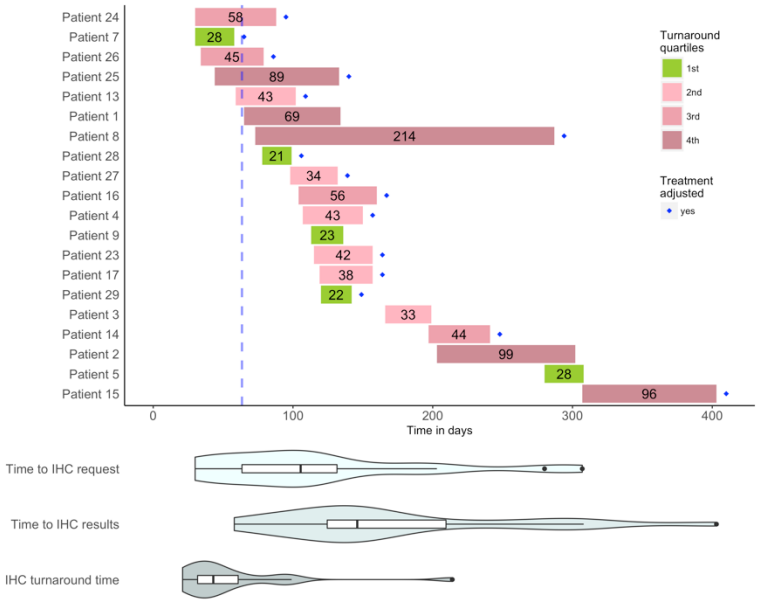


Figure 5. Turnaround times for IHC testing. The size of the bar indicates time in days from requesting results for IHC testing to obtaining them. For each patient, the initial time is the first day of follow-up treatment at the hospital. The color of the bar indicates the quartile within which the data falls in the distribution. The diamond mark indicates that the treatment was adjusted, according to protocols, after obtaining IHC results. The blue dashed line indicates the 1st quartile for the time it took before an IHC request was submitted. Violin plots with whiskers are correspondingly provided for the time to IHC request (from start to request), time to IHC results (from start to the obtaining of results), and turnaround time of IHC testing (from request to results).

Association rules

Association rules were mined from the events of this cohort using the Apriori algorithm. The five top-ranking association rules are shown in Figure 6. These rules have a support of

63.3% of cases, with a confidence of 90.5% and a rule performance (lift, or precision) of 1.3. For rules with lift larger than 1, it can be assumed that the right-hand side of the rule is a consequence of the left-hand side. In our mined rules, we can observe that, in general, IHC testing, particularly ER/PR testing, results in adjusting treatment plans.

Figure 6. Top-5 association rules mined with the Apriori algorithm

4. Discussion

Key findings

Breast cancer in Mexico (and worldwide) continues to be a public health concern, and increasing research is being done on the characterization of better genomic variants. Although our study investigated a small number of patients, the prevalence and epidemiological characteristics among our cohort are similar to what has previously been estimated for the country (i.e. in regards to age group and staging) [10]. Our study further investigates the timeliness of IHC testing, so our conclusions can then be used to inform hospital administrators and public health officials around the world about the importance of timely IHC testing.

306 On the therapeutic value. The therapeutic importance of immunophenotyping (the use of
307 IHC testing for subtyping of cancer patients) needs to be carefully considered and addressed in
308 treatment plans by oncologists. IMSS protocol states that estrogen positive (ER+) and/or
309 progesterone positive (PR+) patients could benefit from hormone replacement therapy to prevent
310 recurrence. For the patient, that usually means that the traditional FEC chemotherapy regimen
311 composed by 5-fluorouracil, epirubicin, and cyclophosphamide, would be adjusted to include
312 some hormone replacement therapy, either aromatase inhibitors to block estrogen production
313 (e.g. anastrozole, letrozole, or exemestane), or some drug that could interfere with the ability of
314 estrogene to stimulate the growth of breast cells (e.g. tamoxifene or toremifene).

315 In contrast, patients with HER2 positive (HER2+), which tumors tend to grow and spread
316 more aggressively, could benefit from a targeted therapy that could prescribe drugs to block
317 HER2 (e.g. trastuzumab or pertuzumab). However, the use of these drugs can have different side
318 effects than traditional chemotherapy. The use of in situ hybridization (ISH), instead of IHC, can
319 be used to determine HER2 status with an overall concordance with IHC, and it may be more
320 beneficial to use both [18]. There is a need to balance sensitivity of the study with hospital
321 workload and expenses. Furthermore, HER2 status has been successfully incorporated into
322 medical practice to guide treatment decisions for breast cancer patients [19]. In fact, the
323 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) updated
324 their 2013 guidelines to designate more patients as eligible for trastuzumab therapy, in
325 accordance with ISH and IHC testing [20].

326 On the timeliness of testing. Opportune IHC testing, understood as the availability of IHC
327 results before the beginning of any treatment, is of critical importance. For IMSS, the timeliness
328 of IHC results were typically not opportune, but whenever they were available, they triggered a

329 response from the clinical oncology team to adjust patient treatments. The widespread
330 distribution of time to request and turnaround time demonstrates the lack of standardization of
331 this process. Many factors could have contributed to the delay in IHC testing, including: patient
332 non-adherence to appointments; hospital overflow, resulting in longer appointments; logistical
333 problems relating to IHC testing's status an external service to the IMSS hospital of this study;
334 and administrative problems in the hospital resulting in inadequate tracking of tests and results.

335 On the need for improved clinical guidelines. It is important to establish an appropriate
336 timeline for opportune IHC testing. For example, In the United States, the College of American
337 Pathologists' (CAP) guideline on turnaround time for standard biopsies is around two days for
338 90% of cases [21]. In fact, the joint guideline on HER2 testing from the American Society of
339 Clinical Oncology (ASCO) and CAP recommends informing the patients about the expected
340 turnaround time [22]. In Australia, a study with more than 78,000 patients tested for HER2
341 status, with both ISH and IHC, revealed that the average turnaround time was between 4-5 days,
342 which decreased due to the increased volume of patients being tested [23]. In Saudi Arabia, a
343 study in 2015 showed that 24% of cases fall outside the recommended CAP turnaround time
344 [24]. The European Society for Medical Oncology (ESMO) published a survey on 24 European
345 countries were the turnaround time was 10 days or less for 89% of laboratories [25]. In the
346 United Kingdom, the Royal College of Pathologists recommend the use of key performance
347 indicators to have a histopathology diagnosis within seven days of biopsy in 90% of cases [26].

348 Given their resource-available settings, clinical guidelines in developed countries do not
349 have a recommendation regarding the maximum turnaround time that could be effective in a
350 patient's treatment trajectory. More importantly, they do not address the time between initial
351 diagnosis, molecular testing (either via IHC or genetics), and the selection of treatment. In

352 Mexico, clinical guidelines for breast cancer recommend testing for ER, PR, and HER2 as part of
353 the histopathology study, but fail to provide guidance regarding turnaround time [13]. IMSS
354 maintains a medical procedures manual, which includes ten key indicators for breast cancer
355 screening, diagnosis, and treatment [27]. This manual measures time-to-diagnosis in a 30-day
356 period, including imaging through mammography and results of histopathology report. In
357 addition, the institution also measures time-to-treatment measured from the date of diagnosis,
358 which should be achieved within a 21-day period. Recognizing the resource constraints of IMSS,
359 the implementation of a key indicator policy related to IHC testing might significantly reduce
360 turnaround times.
361

362 **Limitations**

363 On the need for accurate electronic health records (EHR). Missing information was
364 common across the paper and electronic records in our study. Clinicians and administrative staff
365 at the IMSS hospital are still getting used to the novel implementation of the EHR, which had an
366 impact on our ability to better characterize this cohort. In the absence of a cancer registry, our
367 best estimate of the disease is the institutional census. As the hospitals in Mexico, and elsewhere,
368 continue to become more and more electronic, there is a need to develop better software tools to
369 analyze the information obtained, including medical natural language processing and machine
370 learning applications.

371 On the visual representation. The medical trajectories shown in Figure 1 are useful in
372 quickly providing a patient history overview of hospital care. With more work on the user
373 interface, we envision this tool could eventually represent a valuable visual aid in which a patient
374 and their companions might be able to better communicate with their clinicians about the

375 management of their disease (it could probably also appear in a printed version). Currently, some
376 of the challenges of this tool include the missing identification of events that are overlapping,
377 and the impossibility of further elaborating on the details of each event. For a hospital
378 administrator, Figure 1 can provide a quick overview of the patients seen in their hospital, which
379 could be used as a decision-making tool, with the proper validation. This visual aid creation tool
380 can be obtained from the repository at https://github.com/arturolp/patientTrajectory.git
381

382 **Healthcare system implications**

383 Adverse events in the trajectory of an oncological patient, which might include
384 hospitalization related to cancer, recurrence of tumor, or metastasis, are extremely costly for the
385 healthcare system. The use of IHC testing was shown in our study to help with the selection of
386 precise treatment for patients (either by adjusting the treatment or confirming it). The association
387 rules found in our study confirm that ER and PR testing are associated with the adjustment of
388 treatment. However, the time in which IHC testing is performed is of critical importance if we
389 want to influence improved prognosis.

390 IHC testing is being carried out as an external service at the IMSS hospital in our study.
391 As the Mexican healthcare system continues to transition from reactive to preventative care, the
392 need for more IHC testing in breast cancer and other diseases will certainly allow for the further
393 development of its own testing facilities, therefore allowing for some economy of scale.

394 We have shown that the use of surrogate immunohistochemistry, as described by the
395 Mexican Consensus and previous literature worldwide, can have a beneficial therapeutic effect
396 on breast cancer patients. The aims of any healthcare system should be the identification of
397 earlier events that can have an impact on downstream events in the trajectory of an oncological

398 patient's treatment. In resource-constrained settings, it is important not only to consider surrogate
399 alternatives to more costly diagnostics (e.g. genomic testing), but also to incorporate the
400 regulatory and logistical aspects of implementing these surrogate IHC tests. IMSS must face the
401 important challenge of continuing to improve their turnaround times, which should have a
402 positive impact on the prognosis of their patients. Finally, other healthcare systems would also
403 benefit from determining the maximum turnaround time in which IHC testing can still be
404 opportune and have a therapeutic beneficial effect.
405

406 **Acknowledgements**

407 The authors wish to acknowledge Prof. Ami S. Bhatt, MD, PhD, for her valuable
408 contribution and comments regarding this project. Also to Katie Kanagawa, PhD, for her
409 valuable support in editing this manuscript.
410

411 **Authors' contributions**

412 Conceptualization: MFRM, ALP, and CAA
413 Data curation: MFRM
414 Formal analysis: MFRM and ALP
415 Funding acquisition: CDB
416 Investigation: MRFM, ALP, SMFV, and CAA
417 Methodology: ALP

418 Resources: RAR and CDB

419 Software: ALP

420 Supervision: CDB, RAR, and CAA

421 Visualization: MFRM, ALP

422 Writing – original draft: ALP

423 Writing – review & editing: MFRM, CAA, SMFV, RAR, CDB, ALP

424

425 **Data Availability**

426 Data belongs to the Mexican Institute of Social Security (IMSS), through its state
427 Delegation of Michoacan. IMSS's Coordination of Health Research may grant access to this data
428 on a case-by-case basis to researchers who obtain the necessary approvals from IMSS's National
429 Scientific Research Committee and National Bioethics Committee.

430

431 **Funding**

432 Research reported in this publication was supported by grant 8316sc-7 from the joint
433 Center of Excellence in Regulatory Science and Innovation (CERSI) at the University of
434 California, San Francisco and Stanford University. The funder had no role in study design, data
435 collection and analysis, decision to publish, or preparation of the manuscript.

436

437 **Competing interests**

438 RAR declares to be IMSS's state delegate in Michoacan, overseeing the hospital
439 mentioned in this study. The remaining authors declare no conflicts of interest.
440

441 **References**

442 1. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression
443 patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc
444 Natl Acad Sci U S A. National Acad Sciences; 2001;98: 10869–10874.
445 doi:10.1073/pnas.191367098
446 2. Abu-Khalf M, Pusztai L. Influence of genomics on adjuvant treatments for pre-invasive
447 and invasive breast cancer. Breast. 2013;22 Suppl 2: S83–7.
448 doi:10.1016/j.breast.2013.07.015
449 3. Enewold L, Geiger AM, Zujewski J, Harlan LC. Oncotype Dx assay and breast cancer in
450 the United States: usage and concordance with chemotherapy. Breast Cancer Res Treat.
451 Springer US; 2015;151: 149–156. doi:10.1007/s10549-015-3366-7
452 4. Lips EH, Mulder L, de Ronde JJ, Mandjes IAM, Koolen BB, Wessels LFA, et al. Breast
453 cancer subtyping by immunohistochemistry and histological grade outperforms breast
454 cancer intrinsic subtypes in predicting neoadjuvant chemotherapy response. Breast Cancer
455 Res Treat. Springer US; 2013;140: 63–71. doi:10.1007/s10549-013-2620-0
456 5. Won JR, Gao D, Chow C, Cheng J, Lau SYH, Ellis MJ, et al. A survey of
457 immunohistochemical biomarkers for basal-like breast cancer against a gene expression

458 profile gold standard. Mod Pathol. 2013;26: 1438–1450. doi:10.1038/modpathol.2013.97
459 6. Cardenas Sanchez J, Bargallo Rocha JE, Erazo Valle A, Poitevin Chacon A, Valero
460 Castillo V, Perez-Sanchez V. [Consenso Mexicano sobre diagnóstico y tratamiento del
461 cáncer mamario]. 6 ed. 2015. pp. 1–149.
462 7. Lara-Medina F, Perez-Sanchez V, Saavedra-Perez D, Blake-Cerda M, Arce C, Motola-
463 Kuba D, et al. Triple-negative breast cancer in Hispanic patients: high prevalence, poor
464 prognosis, and association with menopausal status, body mass index, and parity. Cancer.
465 2011;117: 3658–3669. doi:10.1002/cncr.25961
466 8. Robles-Castillo J, Ruvalcaba-Limón E. [Cáncer de mama en mujeres mexicanas menores
467 de 40 años]. Ginecologia y Obstetricia de Mexico. 2011;79: 482–488.
468 9. Villarreal-Garza C, Alvarez-Gomez RM, Perez-Plasencia C, Herrera LA, Herzog J,
469 Castillo D, et al. Significant clinical impact of recurrent BRCA1 and BRCA2 mutations in
470 Mexico. Cancer. 2015;121: 372–378. doi:10.1002/cncr.29058
471 10. Mohar-Betancourt A, Reynoso-Noveron N, Armas-Texta D, Gutierrez-Delgado C, Torres-
472 Dominguez JA. Cancer Trends in Mexico: Essential Data for the Creation and Follow-Up
473 of Public Policies. Journal of Global Oncology. 2017;: JGO.2016.007476–9.
474 doi:10.1200/JGO.2016.007476
475 11. Secretaria de Salud de Mexico. [Diagnóstico y Tratamiento de la Patología Mamaria
476 Benigna en Primer y Segundo Nivel de Atención]. 2009;: 1–66.
477 12. Secretaria de Salud de Mexico. [Prevention, Screening, and Timely Referral of Suspected
478 Breast Cancer in the First Level of Care]. 2011 pp. 1–74.
479 13. Secretaria de Salud de Mexico. [Diagnóstico y Tratamiento del Cáncer de Mama en
480 Segundo y Tercer Nivel de Atención]. 2012;: 1–102.

481 14. Instituto Nacional de Estadística y Geografía. [Encuesta Intercensal 2015]. 2015.
482 15. Parkes J, Hendricks M, Ssenyonga P, Mugamba J, Molyneux E, Schouten van Meeteren
483 A, et al. SIOP PODC adapted treatment recommendations for standard-risk
484 medulloblastoma in low and middle income settings. Pediatric Blood & Cancer.
485 2015;62: 553–564. doi:10.1002/pbc.25313
486 16. Agrawal R, Srikant R. Fast algorithms for mining association rules. Proc 20th int conf
487 very large data bases. 1994.
488 17. Villarreal-Garza C, Weitzel JN, Llacuachaqui M, Sifuentes E, Magallanes-Hoyos MC,
489 Gallardo L, et al. The prevalence of BRCA1 and BRCA2 mutations among young
490 Mexican women with triple-negative breast cancer. Breast Cancer Res Treat. Springer US;
491 2015;150: 389–394. doi:10.1007/s10549-015-3312-8
492 18. Solomon JP, Dell'Aquila M, Fadare O, Hasteh F. Her2/neu Status Determination in Breast
493 Cancer: A Single Institutional Experience Using a Dual-Testing Approach With
494 Immunohistochemistry and Fluorescence In Situ Hybridization. Am J Clin Pathol.
495 2017;147: 432–437. doi:10.1093/ajcp/aqw224
496 19. Goddard KAB, Bowles EJA, Feigelson HS, Habel LA, Alford SH, McCarty CA, et al.
497 Utilization of HER2 genetic testing in a multi-institutional observational study. Am J
498 Manag Care. NIH Public Access; 2012;18: 704–712.
499 20. Lim TH, Lim AST, Thike AA, Tien SL, Tan PH. Implications of the Updated 2013
500 American Society of Clinical Oncology/College of American Pathologists Guideline
501 Recommendations on Human Epidermal Growth Factor Receptor 2 Gene Testing Using
502 Immunohistochemistry and Fluorescence In Situ Hybridization for Breast Cancer. Arch
503 Pathol Lab Med. 2016;140: 140–147. doi:10.5858/arpa.2015-0108-OA

504 21. Novis DA, Zarbo RJ, Saladino AJ. Interinstitutional comparison of surgical biopsy
505 diagnosis turnaround time: a College of American Pathologists Q-Probes study of 5384
506 surgical biopsies in 157 small hospitals. Arch Pathol Lab Med. 1998;122: 951–956.
507 22. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al.
508 Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast
509 Cancer: American Society of Clinical Oncology/College of American Pathologists
510 Clinical Practice Guideline Update. J Clin Oncol. 2013;31: 3997–4013.
511 doi:10.1200/JCO.2013.50.9984
512 23. Morey AL, Brown B, Farshid G, Fox SB, Francis GD, McCue G, et al. Determining
513 HER2 (ERBB2) amplification status in women with breast cancer: final results from the
514 Australian in situ hybridisation program. Pathology. Elsevier; 2016;48: 535–542.
515 doi:10.1016/j.pathol.2016.05.007
516 24. Alshieban S, Al-Surimi K. Reducing turnaround time of surgical pathology reports in
517 pathology and laboratory medicine departments. BMJ Qual Improv Rep. BMJ Open
518 Quality; 2015;4: u209223.w3773. doi:10.1136/bmjquality.u209223.w3773
519 25. Boleij A, Tembuyser L, Taylor A, Kafatos G, Jenkins-Anderson S, Tack V, et al. PD-
520 015A survey on current RAS-mutation testing practices in Europe. Ann Oncol. Oxford
521 University Press; 2015;26: iv105–iv105. doi:10.1093/annonc/mdv234.14
522 26. Pathologists TRCO. Key Performance Indicators in Pathology. 2013;: 1–23.
523 27. Instituto Mexicano del Seguro Social. [Manual Metodológico de Indicadores Médicos
524 2016]. 2016;: 1–452.
525

Cummulative patients with confirmed breast cancer with visits to HGR1 in 2014

(Institutional census)

N = 402

without IHC testing

(Jan 2014 – Mar 2017)

N = 372

Deceased

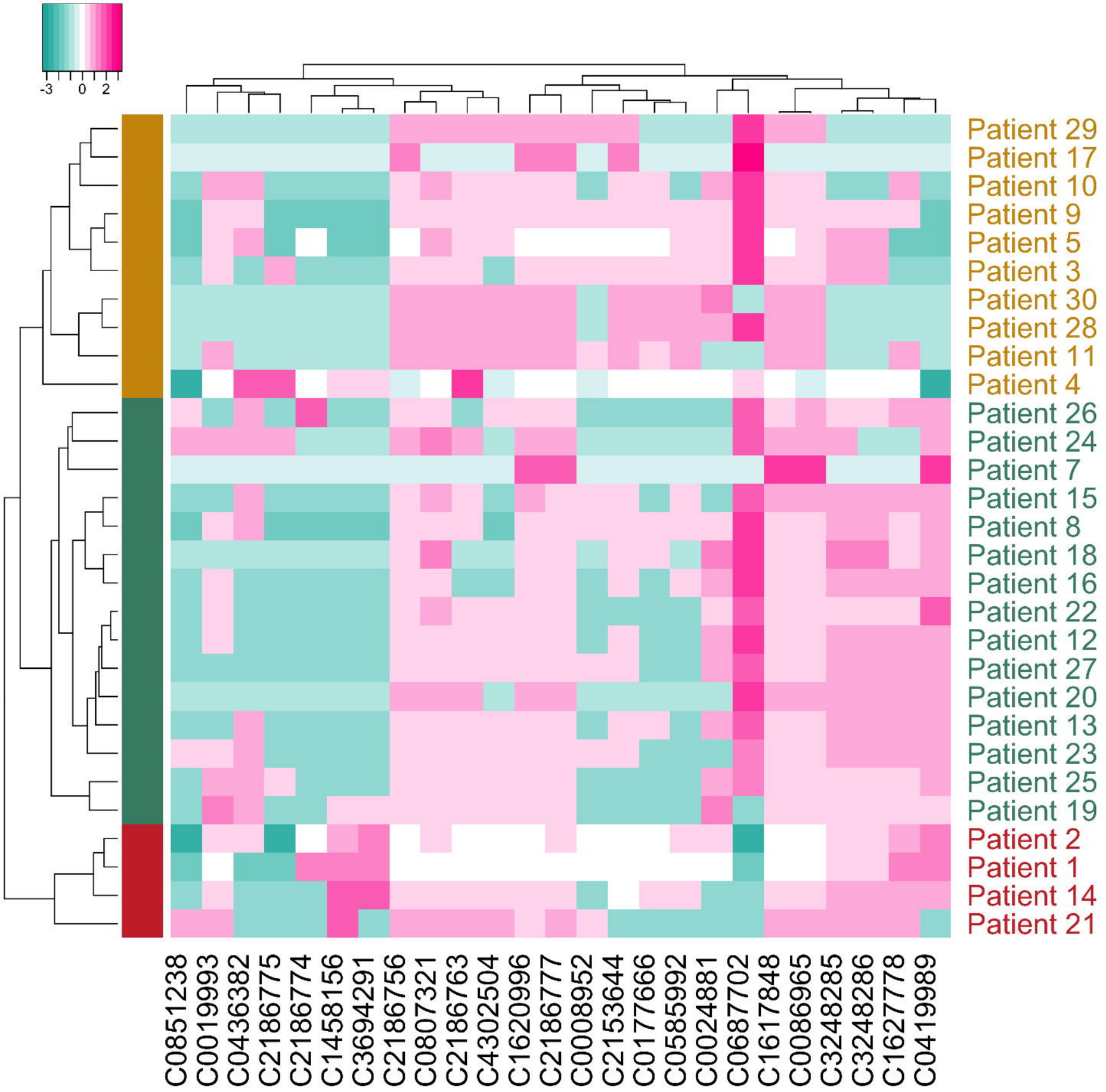
(Jan 2014 – Mar 2017)

N = 0

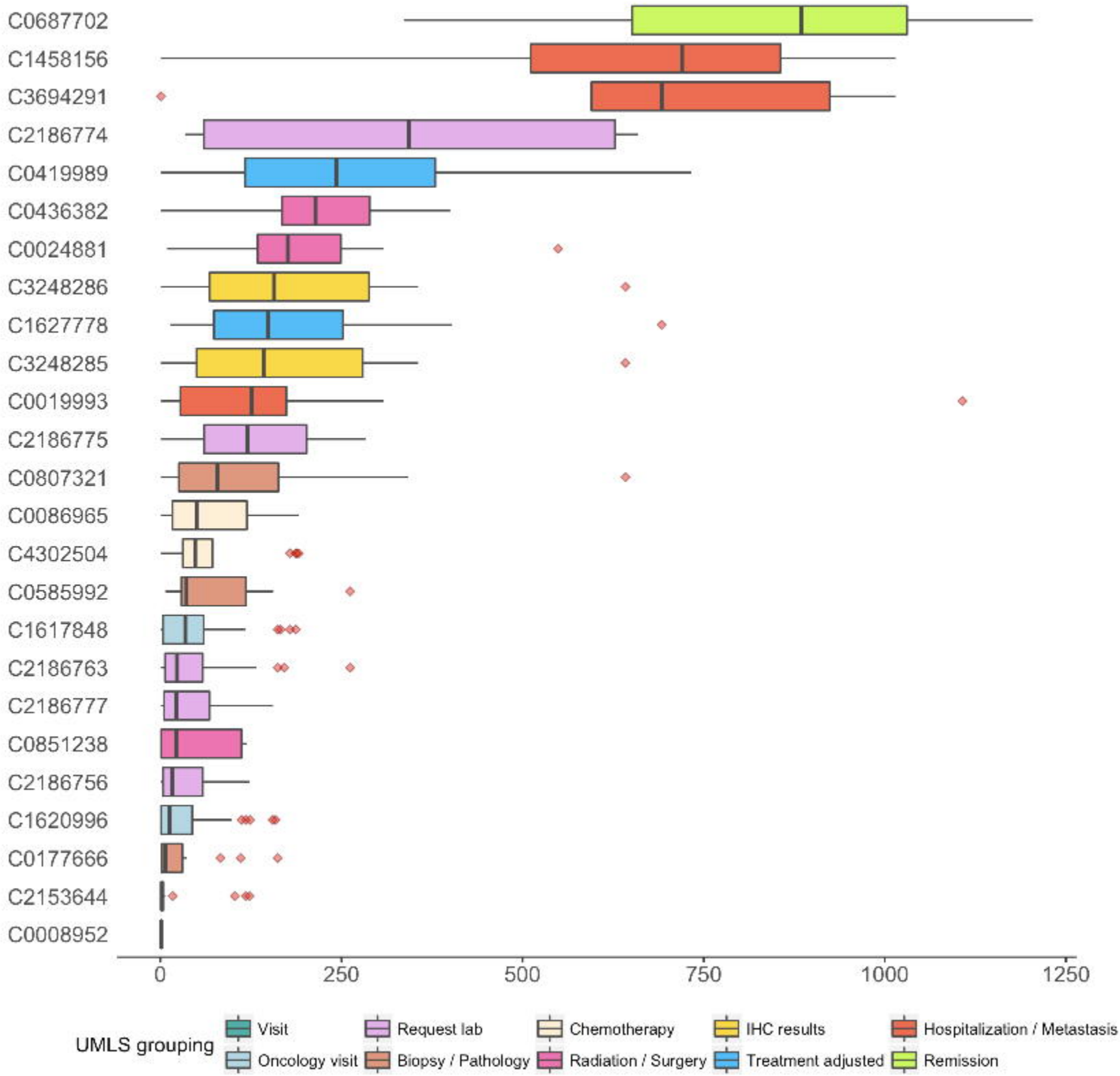
with IHC testing

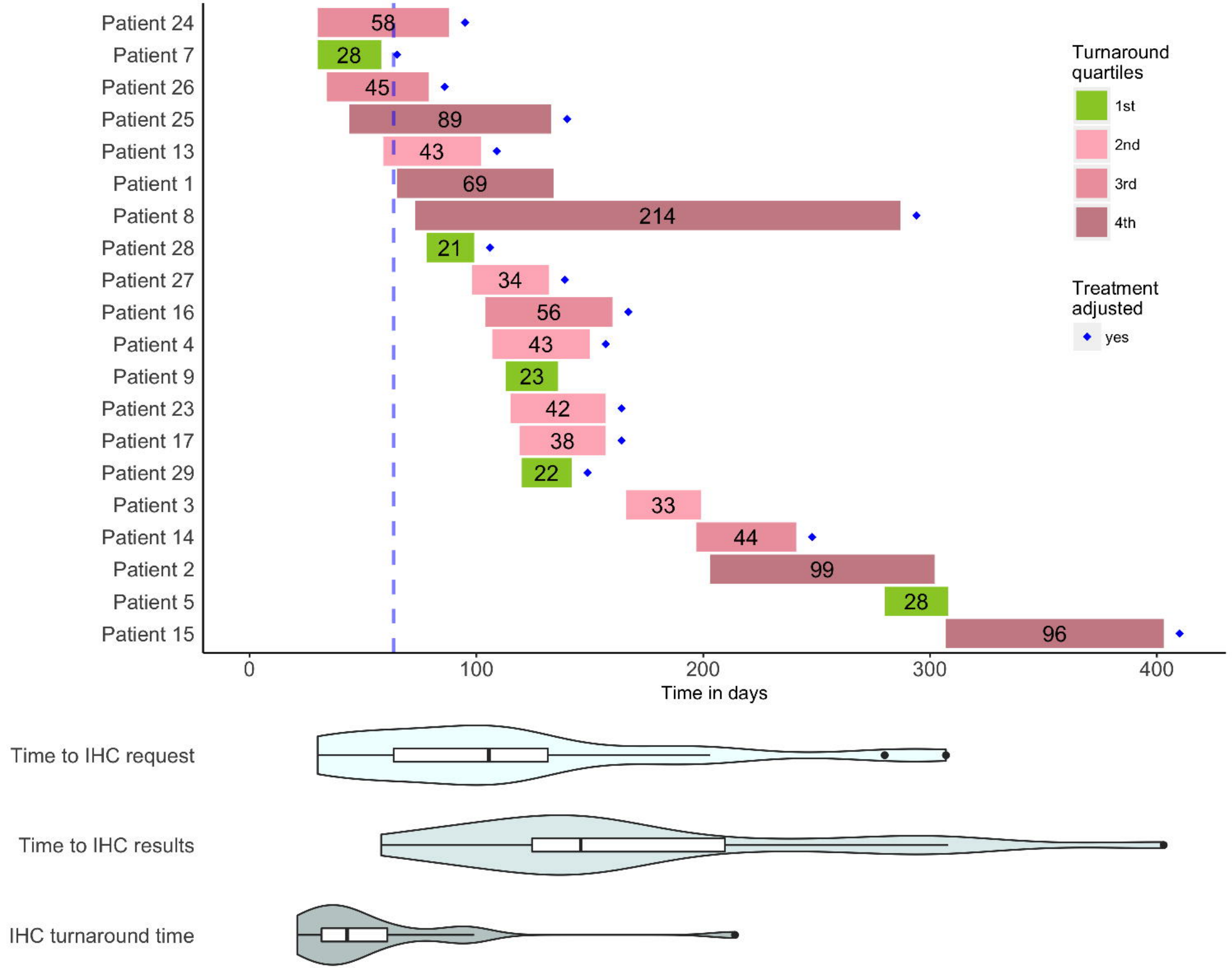
(Jan 2014 – Mar 2017)

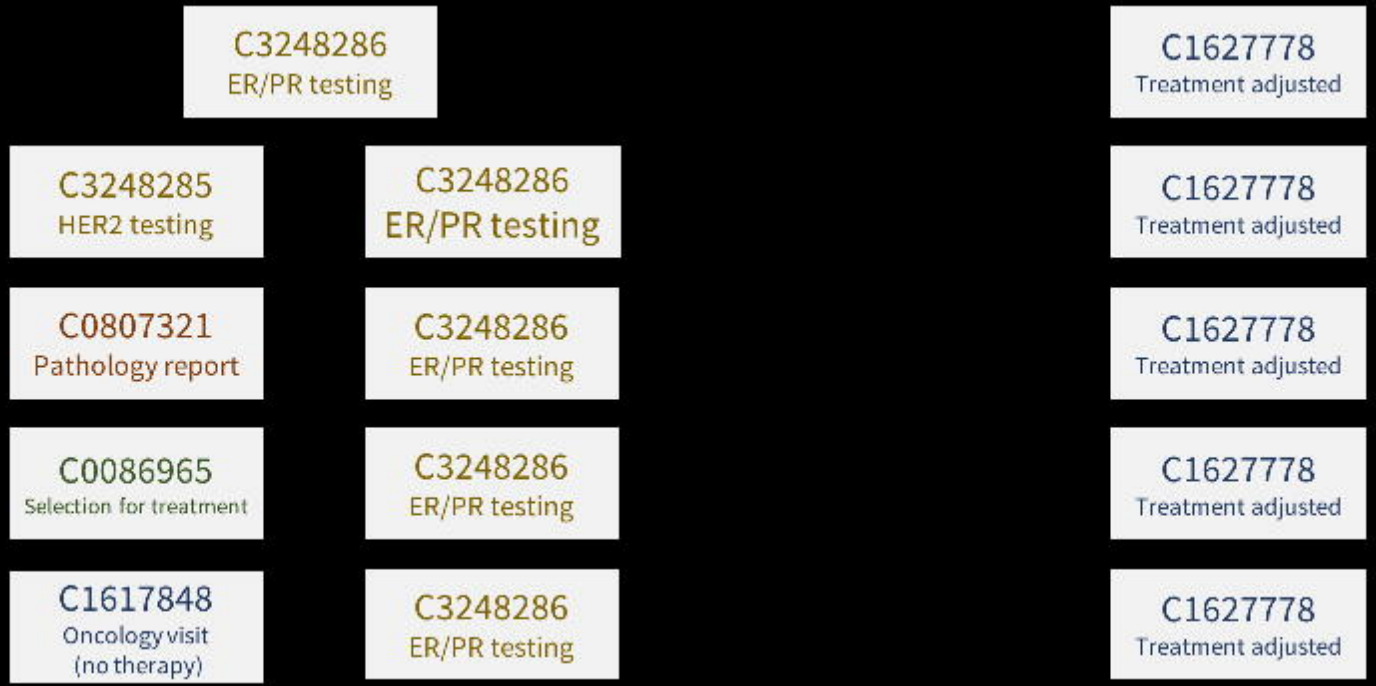
N = 30


-3
0
2
Patient 29
Patient 17
Patient 10
Patient 9
Patient 5
Patient 3
Patient 30
Patient 28
Patient 11
Patient 4
Patient 26
Patient 24
Patient 7
Patient 15
Patient 8
Patient 18
Patient 16
Patient 22
Patient 12
Patient 27
Patient 20
Patient 13
Patient 23
Patient 25
Patient 19
Patient 2
Patient 1
Patient 14
Patient 21
C0851238
C0019993
C0436382
C2186775
C2186774
C1458156
C3694291
C2186756
C0807321
C2186763
C4302504
C1620996
C2186777
C0008952
C2153644
C0177666
C0585992
C0024881
C0687702
C1617848
C0086965
C3248285
C3248286
C1627778
C0419989

Cluster 1
Cluster 2
Cluster 3
Patient 1
Patient 2
Patient 14
Patient 21
Patient 11
Patient 28
Patient 30
Patient 4
Patient 3
Patient 5
Patient 9
Patient 10
Patient 17
Patient 29
Patient 23
Patient 22
Patient 13
Patient 16
Patient 20
Patient 18
Patient 27
Patient 8
Patient 12
Patient 15
Patient 19
Patient 25
Patient 7
Patient 24
Patient 26
UMLS grouping
Visit
Oncology visit
Request lab
Biopsy / Pathology
Chemotherapy
Radiation / Surgery
IHC results
Treatment adjusted
Hospitalization / Metastasis
Remission


C0687702
C1458156
C3694291
C2186774
C0419989
C0436382
C0024881
C3248286
C1627778
C3248285
C0019993
C2186775
C0807321
C0086965
C4302504
C0585992
C1617848
C2186763
C2186777
C0851238
C2186756
C1620996
C0177666
C2153644
C0008952
0
250
500
750
1000
1250
UMLS grouping
Visit
Oncology visit
Request lab
Biopsy / Pathology
Chemotherapy
Radiation / Surgery
IHC results
Treatment adjusted
Hospitalization / Metastasis
Remission


Patient 24
Patient 7
Patient 26
Patient 25
Patient 13
Patient 1
Patient 8
Patient 28
Patient 27
Patient 16
Patient 4
Patient 9
Patient 23
Patient 17
Patient 29
Patient 3
Patient 14
Patient 2
Patient 5
Patient 15
58
28
45
89
43
69
214
21
34
56
43
23
42
38
22
33
44
99
28
96
0
100
200
300
400
Time in days
Turnaround quartiles
1st
2nd
3rd
4th
Treatment adjusted
yes
Time to IHC request
Time to IHC results
IHC turnaround time


C3248286
ER/PR testing
C1627778
Treatment adjusted
C3248285
HER2 testing
C3248286
ER/PR testing
C1627778
Treatment adjusted
C0807321
Pathology report
C3248286
ER/PR testing
C1627778
Treatment adjusted
C0086965
Selection for treatment
C3248286
ER/PR testing
C1627778
Treatment adjusted
C1617848
Oncology visit
(no therapy)
C3248286
ER/PR testing
C1627778
Treatment adjusted