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Novel Immunotherapies in Personalise Cancer Medicine: Oesophageal Gastric Junction Adenocarcinomas and the HER2 Heterogeneity Challenging Results

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Abstract

Cancer heterogeneity is a great challenging factor within the innovative emerging approach in the field of Cancer Precession Medicine. Thus oncologists and pathologists are facing an extraordinary challenge in managing those patients. Cancer heterogeneity is well known in various cancers such as breast and gastric cancers precisely the heterogeneity on human epidermal growth receptor 2 (HER2), our focused gene-mutation case in this study. Unfortunately, HER2 heterogeneity is not well understood yet, but its impact on a considerable number of patients is well documented. Therefore, this study is aiming to investigate the Her2 gene heterogeneity in gastroesophageal cancer patients. Sequential serial tissue sections were examined at different levels/depths for HER2 protein expressions by applying the standard immunohistochemistry (IHC) technique. The sequential serial sections were also used for testing the HER2 gene amplification hyperdization technique (ISH). HER2 status was classified as positive or negative and HER2 heterogeneous or homogenous for each individual histology representative and site examined according to the standard HER2 testing system in use for gastric cancers. There was an obvious variation in Her2 protein expression which was expected, while all blocks have shown also variable Her2 protein intensity scoring, none was amplified as all sections examined scored below (1.8) ratio (HER2 gene: centromere). Our study shows the current routine of HER2 testing of a single test stands a great chance of miss-diagnosis for positive HER2 mutation in GOJ patients. Multiple sections to be tested for negative cases could save lives and therefore, are highly recommended.

Keywords: HER2, Heterogeneity, Gastroesophageal junction cancer, Trustazumab, Immunohistochemistry, and Precision medicine

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1. INTRODUCTION

Oesophageal gastric junction (GOJ) adenocarcinomas are tumours defined by exceeding beyond the approximate gastric folds, also known as oesophageal adenocarcinomas and the later is in dispute. Less often some conditions of the GOJ cancers, Barrett's esophagus is not the underlying cause, where, tumour rose from infiltrating sub-cardiac mucosa [1]. Therefore, OGJ adenocarcinomas are significantly heterogeneous tumours. The overall survival is poorly marked as less than 20% of patients have a 5-year

survival rate. The HER2 gene (185-Kd) is a growth factor membrane receptor gene, a member of the HER-growth factor family and it is located on chromosome 17 [2]. It transmits signals regulating normal cell growth, development, and survival. HER2 is constitutively expressed in various types of epithelium, however, over-expression of Her2 protein increases the receptor dimerisation and activation of the growth signalling pathway [3].

Surely, cancer heterogeneity or intra-tumour heterogeneity is well known in various cancers such as breast, colon, and gastric cancers. Gastric and gastroesophageal cancers are reported to acquire frequent mutations in the HER2 gene estimated in around 25% of all cases [4]. Unfortunately, HER2 heterogeneity is not well understood yet, but certainly, it impacts a considerable number of patients' disease management. This is only technically because laboratory testing occurs often on a selected single block tissue that represents the tumour. Heterogeneity is responsible for the changes and differences between the tested tissue and the rest of the tumour. In naked terms, it's a matter of luck rather than test efficiency and accuracy. It's well accepted that some patients who tested negative on the first time diagnosis returned at a later time with an aggressive disease of HER2 gene tested positive. Eventually causing a lot of a hard time for patients and oncologists with more likely patients won't survive it [5].

The ToGA (a phase 3, open-label, randomised controlled trial) for Trastuzumab (Herceptin) combined chemotherapy versus chemotherapy alone first in 2010 showed effective HER2-positive advanced gastric and gastroesophageal junction adenocarcinomas [6]. Also recently Trastuzumab combined chemotherapy has shown more effective in responding to HER2-positive in GOJ rather than those at a distal gastric locations [7]. Identifying intra-tumour heterogeneity in OGJ tumours for advanced therapies, or molecular-based therapies is a very demanding subject for Gastroenterology oncologists and similarly pathologists. It's crucial to eliminate those patients who would benefit from advanced therapies, for patient care, and for the healthcare system to manage limited budget resources on those high-cost prescriptions. Heterogeneity exists when the cellular population of a tumour shows more than 5% and less than 50% of a cytogenetic test result for the investigated amplified gene (FISH/ISH). While more than 50% of the cellular population is amplified then it's known as amplified tumour tissue. In other words; if one cell detected has e.g. her2/cep17 ratio is 2.2, or more, among 20 other cells with gene count of 1.8 or less, thus the tumour is called heterogeneous tumour.

It has been notably the various results for the same patient biopsy in OGJ cancers. Heterogeneity would not be possible to spot clinically nor on routine H&E staining. Histopathologists have to investigate further biopsy sites to look into it, or multiple biopsies using one or more immunostaining [8]. In this study, we focused on HER2 testing to identify those patients who would have a better survival chance with a combined Trastuzumab and standard chemotherapy in OGJ.

A high concordance rate of HER2 amplification in gastric tumours was seen in an Italian study when multiple tissue sites from samples of the same patient were used. In contrast, reports from other studies showed that unfortunate for patients been diagnosed exclusively on samples from the primary tumours, when they could have benefited from trastuzumab combined therapy.

In pathology settings, the initial investigation to identify heterogeneity is immunohistochemistry (IHC) testing. HER2 test is widely used in breast cancers to select those patients where disease is been driven by the HER2 gene [9]. Patients with a positive test could benefit from Trastuzumab therapy and their disease could be managed effectively. It has been reported that 20-30% of ductal breast cancer is driven by the HER2 gene. However, HER2 heterogeneity in breast cancer is not very common; in contrast, it's most notable in OGJ cancers.

Recently HER2 is been investigated in GI cancers and showed a pretty similar presence as in breast cancer [4]. Therefore, many studies have been conducted to look into standardised methodology for testing and scoring HER2 in GI cancers.

Trastuzumab is humanised monoclonal antibody (clone 4b5) against an extra-cellular epitope of the receptors with a human IgG1 (5+). Trastuzumab combined chemotherapy is a well-established option for patients with HER2 gene positive in advanced gastric or gastroesophageal junction cancers [10]. Trastuzumab combined therapy is a primary cancer disease prescribed treatment rather than in metastatic disease cases, however, it's not been easy to biopsy metastatic disease before treating them in most cases [11]. HER2 expression gene drives cancer more wildly if not carefully diagnosed and metastasis is more often. Indeed HER2 testing results play a major role in clinicians' treatment plans here. Cancer is known as a

disease of heterogeneity, which has a big impact on HER2 result outcomes, particularly in gastric cancer heterogeneity in our discussion.

2. MATERIALS AND METHODS

This study investigated an exesional OGJ tumour mass of (2.5X2.5X11cm), from a patient of 65 years old, who was admitted to the hospital. Histopathology diagnosis concluded the condition as an invasive poorly differentiated adenocarcinoma, intestinal type, predominantly in the lower esophagus with extension across the gastric junction (stage III). Staining for Her2 was requested and showed heterogeneous significance areas with staining intensity scored up to (3+). From the tumour resections, a mass of 2.5cm across and extends to 2.5 from the distal resection margin and 11cm from the proximal resection margin. Tumour was dissected, according to the Royal College of Pathologists UK sample criteria D, into 5 slices. All slices were embedded in 5 mega blocks, this mass was aimed to enclose the whole cancer tumour for this study. A Tumour map was designed and 5 mega blocks were determined to investigate the excision mass to understand the HER2 heterogeneity in OGJ adenocarcinomas. Sections of 3-4 microns were obtained to study cancer morphology and its margins. HER2 gene expression test using IHC was performed on slices from the 5 mega blocks utilizing the VENTANA medical systems machine, Gastric HER2 scored according to the consensus panel recommendations on HER-2 scoring for gastric cancer by the TOGA project. In surgical specimens, no membrane reactivity or membranous staining in <10% of cells was considered as an IHC score of (0), weak⁄barely perceptible membranous reactivity in >10% of cells (or reactivity only in part of their membrane) was considered as an IHC score of (1+), weak to moderate complete or basolateral membranous reactivity in >10% of cells was considered as an IHC score of (2+), moderate to strong complete or basolateral membranous reactivity in >10% of cells was considered as an IHC score of (3+)⁄positive. Biopsy specimens with cohesive IHC (3+) clones were considered positive irrespective of the relative number of immunopositive cells. Scores (3+) were counted as HER-2 positive, and scores (0 and 1+) as HER-2 negative. Tumours with equivocal IHC result score (2+) were further tested for HER-2 amplification by dual in situ hybridisation (DDISH). Scoring for DDISH is similar to Breast amplification scoring.

PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody (PATHWAY HER-2 (4B5), a standard diagnostic protocol was applied. As we are interested to investigate heterogeneity on the whole tumour mass, therefore, we studied the gene amplification by applying the HER2 bright-field, dual colour dual hatpin in-situ hybridization application method (DDISH) [Both HER2 IHC 4B5 and DDISH were supplied by VENTANA, Roche, US]. Serial sections were used for scoring HER2 gene amplification at the same cancer sites that showed high HER2 protein expression in IHC, also all mega slides were scanned for amplification presence.

For HER2 protein expression on IHC, staining intensity of membranous when more than 30% of tumour cells this considered as (3+), less than 30% considered as (2+/equvocal), more than 5% and less than 20% considered as (1+), and when there is no staining or scanty then this considered as negative. While for gene amplification scoring of Her2 gene and chromosome 17centromere counted in 20 cells and the percentage of (HER2 gene count divided by ch.17 count) if the outcome greater than 2.2 then HER2 gene is amplified and if scored less than (1.8) then the amplification was negative.

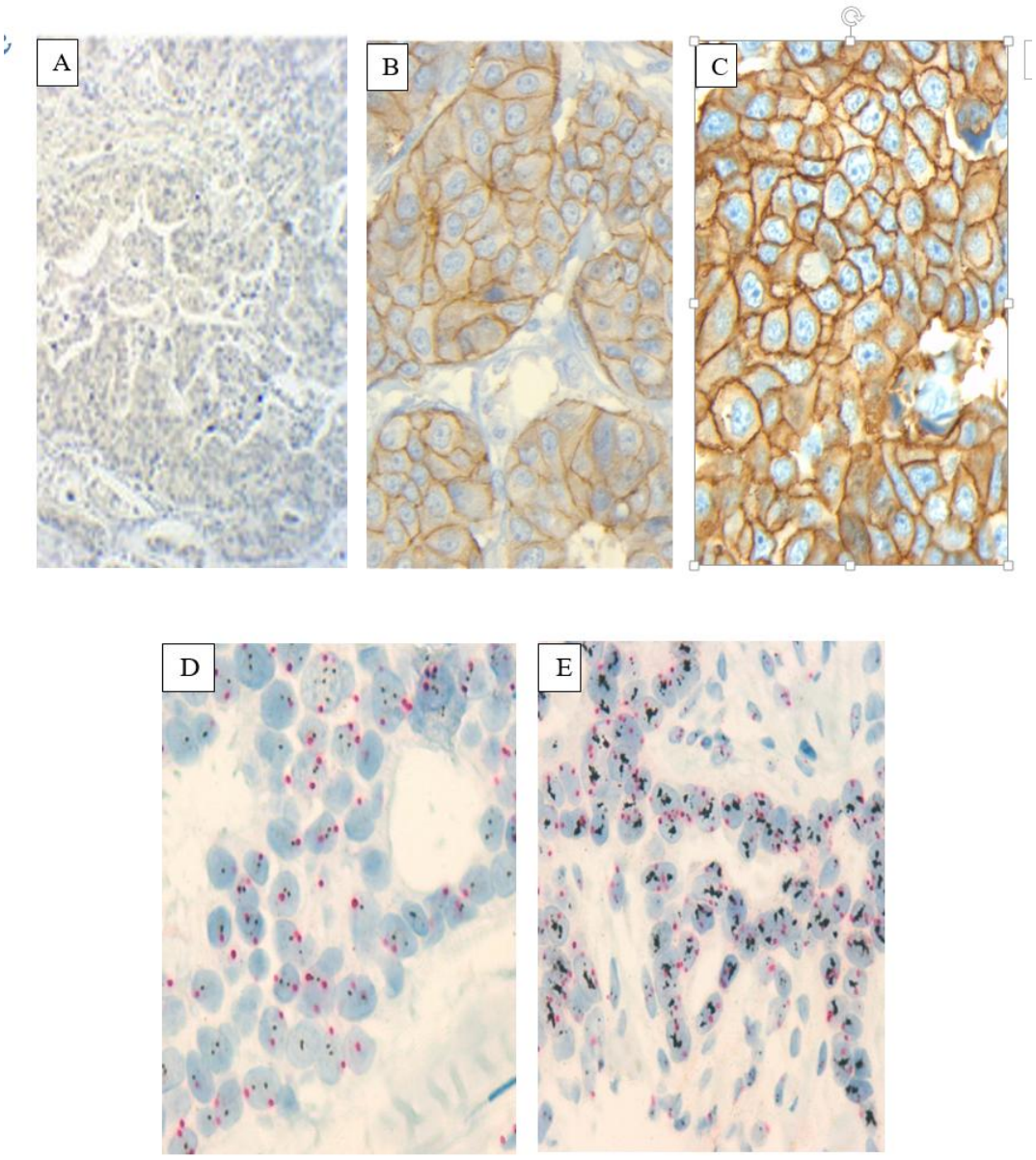

Figure 1: A representative histology findings of Human epidermal growth factor receptor 2 (HER2-IHC) protein expression method in a gastroesophageal tumor. A: A negative (0/1+), case; B: An equivocal (2+) case; C: A positive (3+) case. HER2: Human epidermal growth factor receptor 2 (HER2-ISH) gene expression method, case D: A negative case 1.8. Case; E: A case positive 2.2, and equivocal case considered between (1.8 – 2.2).

3. RESULTS AND DISCUSSIONS

HER2 protein expression was detected in almost all five mega blocks (Figure 1), with obvious variations from site to site on the same tissue section as from block to another one. All five blocks have shown HER2 intensity at (0, 1+, 2+ scores) but only two have shown intensity at (3+) scores. Serial sections studied for HER2 gene amplification results show on all five mega blocks no amplification as all scores were below (1.8). Cellular heterogeneity is well documented in each mega block at least one or 2 cells have scored more than 2.2 when applying the HER2/ch.17 ratio. Chang MC and colleagues 2012 studied 2,522 breast cases and reported the proportion of HER2-amplified nuclei within tumour does not contribute information independent of the actual HER2/ch.17 ratio testing [12]. Their HER2 gene amplification assessment was carried out using the fluorescence in situ hybridization method (FISH), however, we have used in this study the silver in situ hybridisation (SISH) VENTANA Medical Systems method and our results could strengthen their findings. The HER2/ch.17 ratio we have seen here is below the positive recommendation for reporting HER2 positive tumours guidelines, where, this case we studded has shown strong positive HER2 protein

IHC. Certainly, we have studied the whole tumour mass, this would firm the heterogeneity we have reported on HER2 IHC staining has no consequences on the outcomes from the amplification test [13]. Surprisingly, the results at the genetic molecular level, which we thought could explain the heterogeneity we have seen within the HER2 protein IHC has revealed nothing.

At a cellular level heterogeneity was reported in this study on all tumour mega blocks. Where each mega block tumour tissue was processed for DDISH staining and scanned for gene amplification. HER2 protein IHC expression tested locations were identified and scored and no amplification was reported neither at other sites. At least twice per site was scored, and according to the above definition of tumour cellular heterogeneity, the heterogeneous cells were marked [14]. The number of heterogonous cells was divided by the total number of cells scored (20 cells) to calculate the percentage of heterogeneity in each slide. This would flag up a question mark "whether those patients who showed barley cellular heterogeneity would benefit from the trastuzumab therapy or not”? The answer would be a clinical trial and this could bridge the gap between the IHC and ISH/FISH testing missing positive patients.

4.CONCLUSION

Detection of the Her2 gene is very dependent on the particular site of tissue examined. The challenge only can be overcomes by multiple site examination of the tumour mass which is highly recommended for not missing the chance of patients’ eligibility for Trastuzumab novel therapy. The gene amplification counterpart test for Her2 IHC, gene hyperdization, might not be of great benefit, thus the chance of miss-diagnosing Her2 protein presence is high due to the well-known heterogenous nature of the condition

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AUTHOR’S CONTRIBUTION

Zakaria Eltahir conducted all research work, nurtured the main research idea and management of the article, data collection and analysis, writing and final proofreading.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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