The risk of weekend biopsy: Impact of specimen source and fixation status on HER2 assessment in the treatment of advanced gastric cancer (The HER_WEEKEND study)

Michio Nakamura, Ayako Watanabe, Aki Yoshizawa, Sari Iwasaki, Asako Nomura, Mariko Matsumura, Taichi Murai, Kazufumi Itaya, Yuta Koike, Takaaki Izumi, Ayana Endo, Shin Kato, Yuji Ono, Takahiro Ohshima, Nanase Okazaki, Shimpei Nakagawa, Yasushi Ishii, Yuichiro Fukasawa, Isao Yokota, Takahiro Tsuji, Shuji Nishikawa

[要約]

背景；

　胃癌においてヒト上皮成長因子受容体 2 型（HER2）の発現を正確に評価することは、化学療法のレジメンの決定に重要である。しかし、ホルマリンによる検体の固定状態が、HER2 の評価の信頼性に影響を及ぼすことが知られている。筆者らは切除不能の進行ないし再発胃癌において、検体採取日（平日 vs 週末）と検体（生検検体 vs 外科的切除検体)の違いによる HER2 発現の評価の相関を明らかにすることを目的とした。

対象と方法；

2008 年 1 月 1 日から 2021 年 12 月 31 日までに、札幌市立総合医療センターで化学療法を受けた原発胃癌患者 177 人（男性 109 人、女性 68 人)を対象とし、

HER2 の陽性率を評価した。ホルマリンによる固定時間は、固定開始から自動包埋処理装置による包埋の開始時間までと定義した。HER2 の発現は免疫組織化学（IHC)および蛍光 in-situ ハイブリダイゼーション（FISH)にて確認した。HER2 陽性は IHC スコア 2＋/FISH 陽性または IHC スコア 3＋と定義し、HER2 陰性は IHC スコア 0 または 1＋または IHC スコア 2＋/FISH 陰性と定義した。

結果；

全体の HER2 陽性率は 18.1%で、週末（12.8%）に比べ平日（20.0%)の陽性率が高かった。生検検体の陽性率は平日（23.9%)が高く、休日（11.1%)は手術検体よりも発現率が低かった。ホルマリン固定時間は、生検、手術検体ともに平日と休日で有意差が認められた。

[Take home massage]

・ホルマリン固定時間が長いと、特に生検検体において HER2 発現を過小評価する可能性がある。週末に採取された検体が週明けに処理される場合は注意が必要である。

・American Society for Clinical Pathology のガイドラインでは、検体採取後

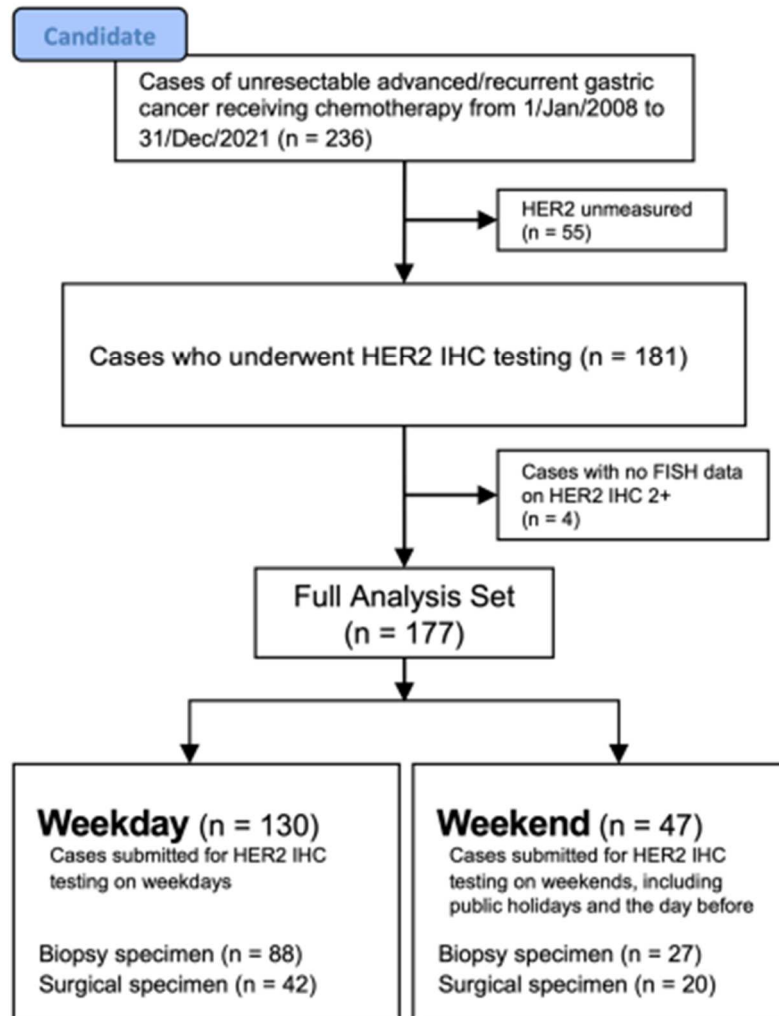10%緩衝ホルマリンに 1 時間以内に浸漬し、6~72 時間急速固定することが推

奨されている。

Figure 1.

FIGURE 1 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) flow chart of patient enrollment. HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

Table 1.

TABLE 1 Baseline characteristics in the entire cohort and in cases on weekdays or weekends.

	Total (N = 177)	Weekday (N = 130)	Weekend (N = 47)	p[*]
Sex, n (%)				
Male	109 (61.6)	83 (63.8)	26 (55.3)	0.30
Female	68 (38.4)	47 (36.2)	21 (44.7)	
Age, median years (range)	68.0 (21–88)	68.0 (21–88)	68.0 (30–83)	0.75[a]
<65 years, n (%)	66 (37.3)	49 (37.7)	17 (36.2)	0.85
≥65 years, n (%)	111 (62.7)	81 (62.3)	30 (63.8)	
Sample source, n (%)				
Biopsy specimen	115 (65.0)	88 (67.7)	27 (57.4)	0.21
Surgical specimen	62 (35.0)	42 (32.3)	20 (42.6)	
HER2 status				
Negative	145 (81.9)	104 (80.0)	41 (87.2)	0.27
Positive	32 (18.1)	26 (20.0)	6 (12.8)	
IHC 0	105 (59.3)	73 (56.2)	32 (68.1)	
IHC 1+	25 (14.1)	19 (14.6)	6 (12.8)	
IHC 2+/FISH−	15 (8.5)	12 (9.2)	3 (6.4)	
IHC 2+/FISH+	9 (5.1)	6 (4.6)	3 (6.4)	
IHC 3+	23 (13.0)	20 (15.4)	3 (6.4)	
FXT, median hours (range, n)	30.4 (2.4–286.5, 168)	28.6 (2.4–240.0, 127)	73.3 (5.1–286.5, 41)	<0.001[a]
Biopsy specimen	22.4 (2.4–125.2, 108)	7.8 (2.4–49.7, 86)	25.4 (5.1–125.2, 22)	0.009[a]
Surgical specimen	56.3 (19.7–286.5, 60)	46.3 (19.7–240.0, 41)	94.6 (62.0–286.5, 19)	<0.001[a]
FXT per MLA, median hours (range, n)	2.2 (0.2–50.1, 165)	2.2 (0.2–30.7, 126)	3.0 (0.7–50.1, 39)	0.048[a]
Biopsy specimen	4.9 (0.8–50.1, 107)	2.8 (0.8–30.7, 86)	8.6 (1.1–50.1, 21)	0.013[a]
Surgical specimen	0.9 (0.2–4.8, 58)	0.7 (0.2–3.5, 40)	1.6 (0.7–4.8, 18)	0.002[a]
Formalin concentration, n (%)				
10%	59 (33.3)	41 (31.5)	18 (38.3)	0.40
20%	118 (66.6)	89 (68.5)	29 (61.7)	
Tumor location: major site, n (%)				
U	51 (28.8)	37 (28.5)	14 (29.8)	0.96[b]
M	77 (43.5)	57 (43.8)	20 (42.6)	
L	48 (27.1)	36 (27.7)	12 (25.5)	
Unknown	1 (0.6)	0 (0)	1 (2.1)	
Lauren's classification, n (%)				
Intestinal	75 (42.4)	58 (44.6)	17 (36.2)	0.28[b]
Diffuse	100 (56.5)	70 (53.8)	30 (63.8)	
Unclassified/no data	2 (1.1)	2 (1.5)	0 (0)	
Peritoneal metastasis, n (%)				
P0	122 (68.9)	88 (67.7)	34 (72.3)	0.56
P1	55 (31.1)	42 (32.3)	13 (27.7)	

	Total (N = 177)	Weekday (N = 130)	Weekend (N = 47)	p[*]
Hepatic metastasis, n (%)				
H0	128 (72.3)	96 (73.8)	32 (68.1)	0.45
H1	49 (27.7)	34 (26.2)	15 (31.9)	

Note: Baseline characteristics of enrolled patients in the HER_WEEKEND study.

Abbreviations: FISH, fluorescence in-situ hybridization; FXT, formalin fixation time; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; L, the lower third of the stomach; M, the middle third of the stomach; MLA, maximum long axis; U, the upper third of the stomach.

[*]χ^2 test.

[a]Student t test.

[b]Excluding unknown/unclassified/no data cases.

Table 2.

TABLE 2 HER2 status comparison.

	N	HER2 positive, N (%)	HER2 negative, N (%)	p*
Overall	177	32 (18.1)	145 (81.9)	
Sample collection day, n (%)				
Weekday	130 (73.4)	26 (20.0)	104 (80.0)	0.27
Weekend	47 (26.6)	6 (12.8)	41 (87.2)	
Sample source for HER2_IHC				
Biopsy specimen, n (%)	115 (65.0)	24 (20.9)	91 (79.1)	0.19
Surgical specimen, n (%)	62 (35.0)	8 (12.9)	54 (87.1)	
Sample source on weekdays				
Biopsy specimen, n (%)	88 (67.7)	21 (23.9)	67 (76.1)	0.11
Surgical specimen, n (%)	42 (32.3)	5 (11.9)	37 (88.1)	
Sample source on weekends				
Biopsy specimen, n (%)	27 (57.4)	3 (11.1)	24 (88.9)	0.69
Surgical specimen, n (%)	20 (42.6)	3 (15.0)	17 (85.0)	
Formalin concentration				
10%	59 (33.3)	11 (18.6)	48 (81.4)	0.89
20%	118 (66.6)	21 (17.8)	97 (82.2)	
Tumor location: major site				
U	51 (28.8)	11 (21.6)	40 (78.4)	0.76[a]
M	77 (43.5)	13 (16.9)	64 (83.1)	
L	48 (27.1)	8 (16.7)	40 (83.3)	
Unknown	1 (0.6)	0 (0)	1 (100)	
Lauren classification, n (%)				
Intestinal	75 (42.4)	22 (29.3)	53 (70.7)	<0.001[a]
Diffuse	100 (56.5)	10 (10.0)	90 (90.0)	
Others	2 (1.1)	0 (0)	2 (100)	
Peritoneal metastasis, n (%)				
P0	122 (68.9)	22 (18.0)	100 (82.0)	0.98
P1	55 (31.1)	10 (18.2)	45 (81.8)	
Hepatic metastasis, n (%)				
H0	128 (72.3)	20 (15.6)	108 (84.4)	0.17
H1	49 (27.7)	12 (24.5)	37 (75.5)	

Abbreviations: HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; L, the lower third of the stomach; M, the middle third of the stomach; U, the upper third of the stomach.

*χ^2 test.

[a]Excluding unknown or unclassified cases.
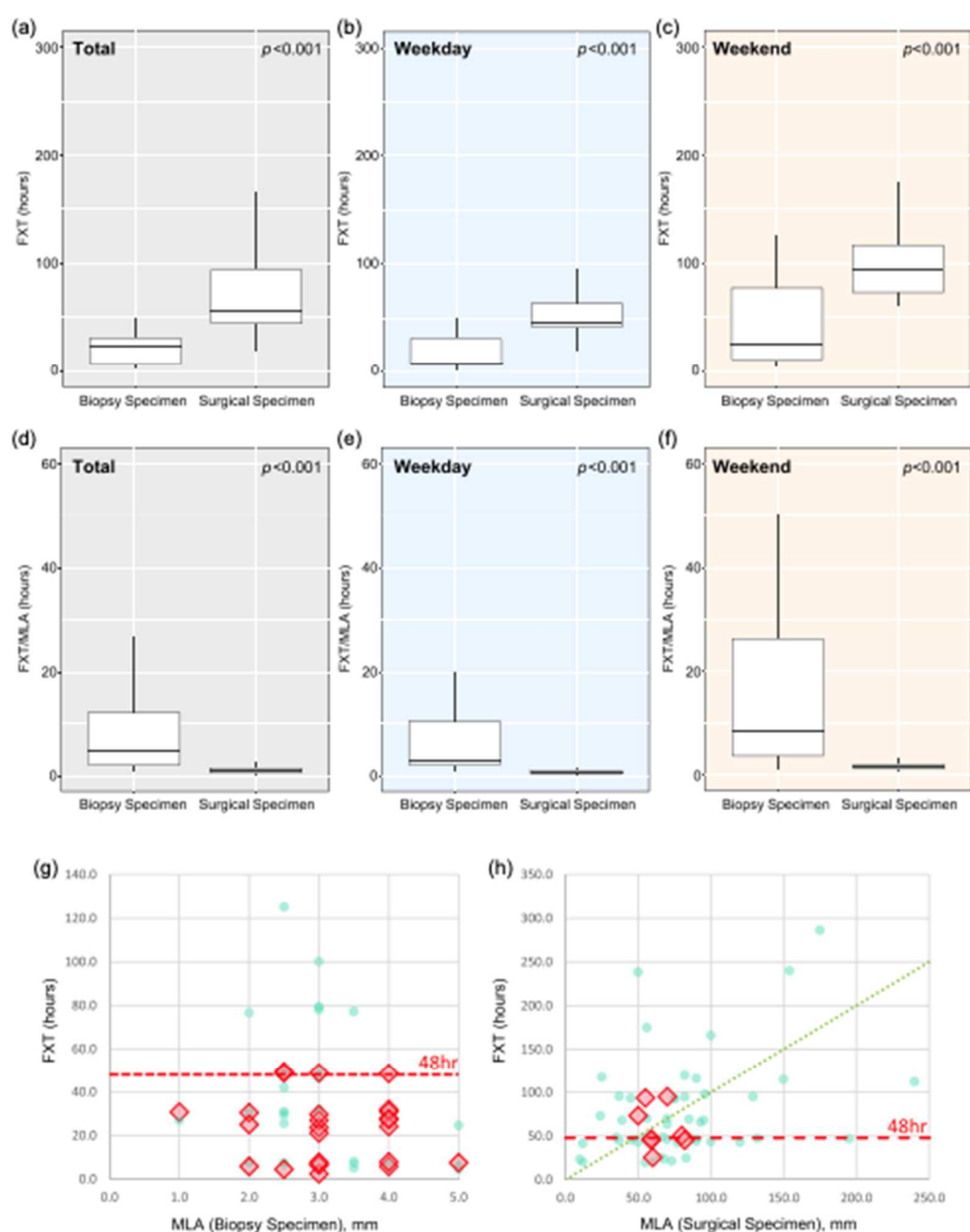
Figure 2.

FIGURE 2 HER2 (human epidermal growth factor receptor 2) positivity and formalin fixation time (FXT) in biopsy (Bx) and surgical specimen (SS) samples. Comparison of FXT between Bx and SS in (a) the overall population, (b) weekdays, and (c) weekends. Comparison of the FXT divided by the maximum long axis of samples (FXT/MLA) between Bx and SS in (d) the overall population, (e) weekdays, and (f) weekends. Differences between Bx and SS in each subgroup were evaluated using Student's *t* test. The red dot represents HER2 positive cases. The green dot represents the FXT corresponding to each MLA of (g) Bx and (h) SS. The red diamond represents HER2 positive cases. The broken red lines indicate the threshold time of 48 h, which is the recommended FXT for HER2 evaluation. The broken green lines show the permeation time of 1 h/mm in 10% neutral buffered formalin.

Table 3.

TABLE 3 Formalin fixation time and ischemic time on HER2 status.

	Total		HER2 positive		HER2 negative		
	N	Median (Range)	n	Median (Range)	n	Median (Range)	p*
FXT, hours (range)	168[a]	30.4 (2.4–286.5)	32	27.1 (2.4–95.0)	136[a]	30.5 (4.5–286.5)	0.023
Biopsy specimen	108[b]	22.4 (2.4–125.2)	24	22.4 (2.4–49.4)	84[b]	16.1 (4.5–125.2)	0.497
Surgical specimen	60[c]	56.3 (19.7–286.5)	8	48.6 (25.5–95.0)	52[c]	62.8 (19.7–286.5)	0.409
MLA, mm (range)	166[d]	3.0 (1–240)	32	3.5 (2–82)	134[d]	3.0 (1–240)	0.032
Biopsy specimen	107[e]	3.0 (1–5)	24	3.0 (2–5)	83[e]	3.0 (1–5)	0.091
Surgical specimen	59[f]	70.0 (10–240)	8	59.5 (50–82)	51[f]	75 (10–240)	0.045
FXT/MLA, hours (range)	165[g]	2.2 (0.2–50.1)	32	2.3 (0.4–19.8)	133[g]	2.1 (0.2–50.1)	0.615
Biopsy specimen	107[e]	4.9 (0.8–50.1)	24	6.9 (0.8–19.8)	83[e]	3.9 (0.9–50.1)	0.147
Surgical specimen	58[h]	0.9 (0.2–4.8)	8	0.8 (0.4–1.7)	50[h]	0.9 (0.2–4.8)	0.469
Ischemic time, mean hours (range)							
Surgical specimen	59[f]	4.0 (0–9.4)	8	3.8 (1.9–6.4)	51[f]	4.1 (0–9.4)	0.837

Abbreviations: FXT, formalin fixation time; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; MLA, maximum long axis.

*Student's *t* test.

[a]Calculated excluding nine cases that could not be measured.
[b]Calculated excluding seven cases that could not be measured.
[c]Calculated excluding two cases that could not be measured.
[d]Calculated excluding 11 cases that could not be measured.
[e]Calculated excluding eight cases that could not be measured.
[f]Calculated excluding three cases that could not be measured.
[g]Calculated excluding 12 cases that could not be measured.
[h]Calculated excluding four cases that could not be measured.