

The value of serum p53 antibody as a biomarker in oral and pharyngeal squamous cell carcinoma

Shotaro Hirokawa, MD¹, Koji Araki, MD, PhD¹, Taku Yamashita, MD, PhD², Hideaki Shimada, MD, PhD³, Masayuki Tomifuji, MD, PhD¹, Kosuke Uno, MD, PhD¹, Akihiro Shiotani, MD, PhD¹

¹: Department of Otorhinolaryngology-Head and Neck Surgery, National Defense Medical College, Tokorozawa, Saitama 359-8513, Japan

²: Department of Otorhinolaryngology, Head and Neck Surgery, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagami-hara, Kanagawa 252-0374, Japan.

³: Department of Surgery, School of Medicine, Toho University Omori Medical Center, Tokyo 143-8541, Japan



Abstract

Objective: To determine whether serum p53 antibody (s-p53-Ab) could be used as a new biomarker for patients with oral and pharyngeal cancer.

Materials and methods: Seventy-one patients newly diagnosed with oral and pharyngeal carcinoma and 117 healthy volunteers or patients with benign head and neck disease were enrolled to this study. Pre-treatment serum was collected for patients and controls and s-p53-Ab, squamous cell carcinoma (SCC)-antigen, and cytokeratin 19 fragment (CYFRA21-1) levels were analyzed. Patients were treated according to standard malignancy protocols and received regular follow-ups.

Results: Comparing the distribution of s-p53-Ab titers, significant differences were observed between the SCC and control groups ($p < 0.05$). Using 1.3 U/mL as the cut-off value for s-p53-Ab, 14 of 69 patients (sensitivity 20%), and 12 of 117 control cases were positive for s-p53-Ab (specificity 89.7%). All nine cases of p16-positive oropharyngeal cancer and three cases of nasopharyngeal cancer were negative for s-p53-Ab. When these cases were excluded, the positive rate in early-stage 1–2 cases was 30%, which was higher than that of SCC-Ag (14%) and CYFRA21-1 (0%). In the analysis of overall and disease-specific survival, s-p53-Ab positivity was not significantly associated with prognosis.

Conclusions: The s-p53-Ab was not detected in any cases of virus-related cancer in which p53 gene mutations were not involved in carcinogenesis. The p53 gene mutation is strongly associated with carcinogenesis in oral, p16- oropharyngeal, and hypopharyngeal cancer. Since the mutant p53 protein is likely to appear in these tumors, it is considered that the s-p53-Ab positive rate is high even in early-stage disease. Thus, the s-p53-Ab measurement may be useful as an early diagnostic biomarker in patients with malignant tumors at these sites.

Introduction

- Early detection is the key of treatment for **head and neck squamous cell carcinoma (HNSCC)** patients.
- The development of sensitive biomarkers for the detection of early-stage HNSCC would improve cancer screening and post-treatment follow-up.
- There is great interest in developing liquid biomarkers as monitoring tools that enable early diagnosis while reducing patient burden.
- The **p53 gene** controls the suppression of cell growth cycles (DNA repair, cell growth arrest, apoptosis) , and known as a tumor suppressor gene and most frequently mutated in malignant tumors including HNSCC.
- Overexpression of mutant p53 induce **serum p53 antibody (s-p53-Ab)** and can be detected in the serum, and it is expected to be useful as biomarker related to diagnosis and prognosis in several reports about various malignant tumors.
- We assessed the value of s-p53-Ab as a new cancer biomarker in patients with HNSCC.**

Materials & Methods

Participants

SCC patients	Exclusion criteria:
71 patients newly diagnosed with oral and pharyngeal carcinoma	
Controls	
117 healthy volunteers and patients with benign head and neck disease	

- Patients with HNSCC other than oral and pharyngeal carcinoma
- Other malignant disease within 5-years
- The following history in the last 12 months
 - Blood transfusion
 - Radiotherapy
 - Chemotherapy

Blood sample analysis

The blood samples were collected from participants before treatment.

Samples were centrifuged at 5000 × g for 5 min, then serum was collected.

The following serum biomarkers were measured by each method.

Serum p53 antibody (s-p53-Ab)

- Enzyme-linked immunosorbent assay (ELISA; MESACUP anti-p53 Test; Medical and Biological Laboratories Co. Ltd., Nagoya, Japan)

SCC antigen (SCC-Ag)

- Automatic chemiluminescence immunoassay analyzer (ARCHITECT i2000; Abbott Japan, Tokyo, Japan)

Cytokeratin 19 fragment (CYFRA21-1)

- Automated chemiluminescent immunoassay system (Lumipulse Presto; FUJIREBIO Inc., Tokyo, Japan)

Results

		s-p53-Ab (U/mL)			SCC-Ag (ng/mL)			CYFRA21-1 (ng/mL)		
		≥ 1.30	< 1.30	P	≥ 1.5	< 1.5	P	≥ 3.5	< 3.5	P
Patients	Total	14	57		22	39		9	50	
	Gender									
	Male	13	44	0.2740	18	30	0.7533	7	40	1
	Female	1	13		4	9		2	10	
	Age (y)									
	< 65	6	21	1	9	16	1	2	22	0.4441
	≥ 65	8	33		12	21		6	26	
	Primary site									
	Oral	1	10		5	4		0	9	
	Nasopharynx	0	3		1	2		1	2	
T	Oropharynx (p16+)	0	7		3	3		1	5	
	Oropharynx (p16-)	3	6		3	5		2	6	
	Hypopharynx	9	28		8	23		3	26	
	Primary unknown	1	3		2	2		2	2	
	T1-2	7	25	0.7684	8	18	0.5914	3	23	0.7176
	T3-4	7	32		14	21		6	27	
	N									
	N0	7	10	0.0307	3	10	0.3424	1	11	0.6697
	N1-3	7	47		19	29		8	39	
	M									
	M0	13	56	0.3577	20	39	0.1262	8	49	0.2840
	M1	1	1		2	0		1	1	
Clinical stage	I-II	3	12	1	3	9	0.5092	0	12	0.1814
	III-IV	11	45		19	30		9	38	
	Smoking									
	< 40	6	42	0.0523	15	27	1	5	35	0.4503
	(Pack year) ≥ 40	8	15		7	12		4	15	
	Alcohol									
	< 20	8	22	0.2391	11	17	0.7897	4	24	1
	≥ 20	6	35		11	22		5	26	
	Controls									
	Total	12	105		25	92		8	109	
Gender	Male	5	49	0.7710	20	34	0.0002	5	49	0.4681
	Female	7	56		5	58		3	60	
	Age (y)									
	< 65	8	53	0.3673	13	48	1	2	59	0.1501
	≥ 65	4	52		12	44		6	50	

Table 1. Clinical variables in patients and control cases.

- Patients with **p16-negative oropharyngeal and hypopharyngeal cancers (non-virus-related pharyngeal cancer)** had a **higher positive rate for s-p53-Ab**.
- Patients with **nasopharyngeal and p16-positive oropharyngeal cancers (virus-related pharyngeal cancer)** were **all negative** for s-p53-Ab.

Biomarkers	Sensitivity	Specificity	P Value	OR	95% CI
s-p53-Ab	23.0% (14/61)	89.7% (105/117)	0.0271	2.6064	1.1204-6.0633
SCC-Ag	34.6% (18/52)	78.6% (92/117)	0.0852	1.9482	0.9461-4.0120
CYFRA 21-1	14.0% (7/50)	93.2% (109/117)	0.1491	2.2180	0.7577-6.4925
P53 and/or SCC	47.5% (29/61)	70.1% (82/117)	0.0223	2.1232	1.1200-4.0251
P53 and/or CYFRA	31.1% (19/61)	82.1% (96/117)	0.0583	2.0680	1.0079-4.2433
SCC and/or CYFRA	42.3% (22/52)	74.4% (87/117)	0.0462	2.1267	1.0676-4.2362
Any one	52.5% (32/61)	65.8% (77/117)	0.0241	2.1241	1.1299-3.9932

Table 2. Diagnostic accuracy of biomarkers for oral and non-virus-related pharyngeal cancer.

- Compared to 117 controls, **s-p53-Ab was significantly higher in 61 patients with oral and non-virus-related pharyngeal cancer**.
- Combined with conventional biomarkers, s-p53-Ab is more useful in diagnosing oral and non-virus-related pharyngeal cancer.

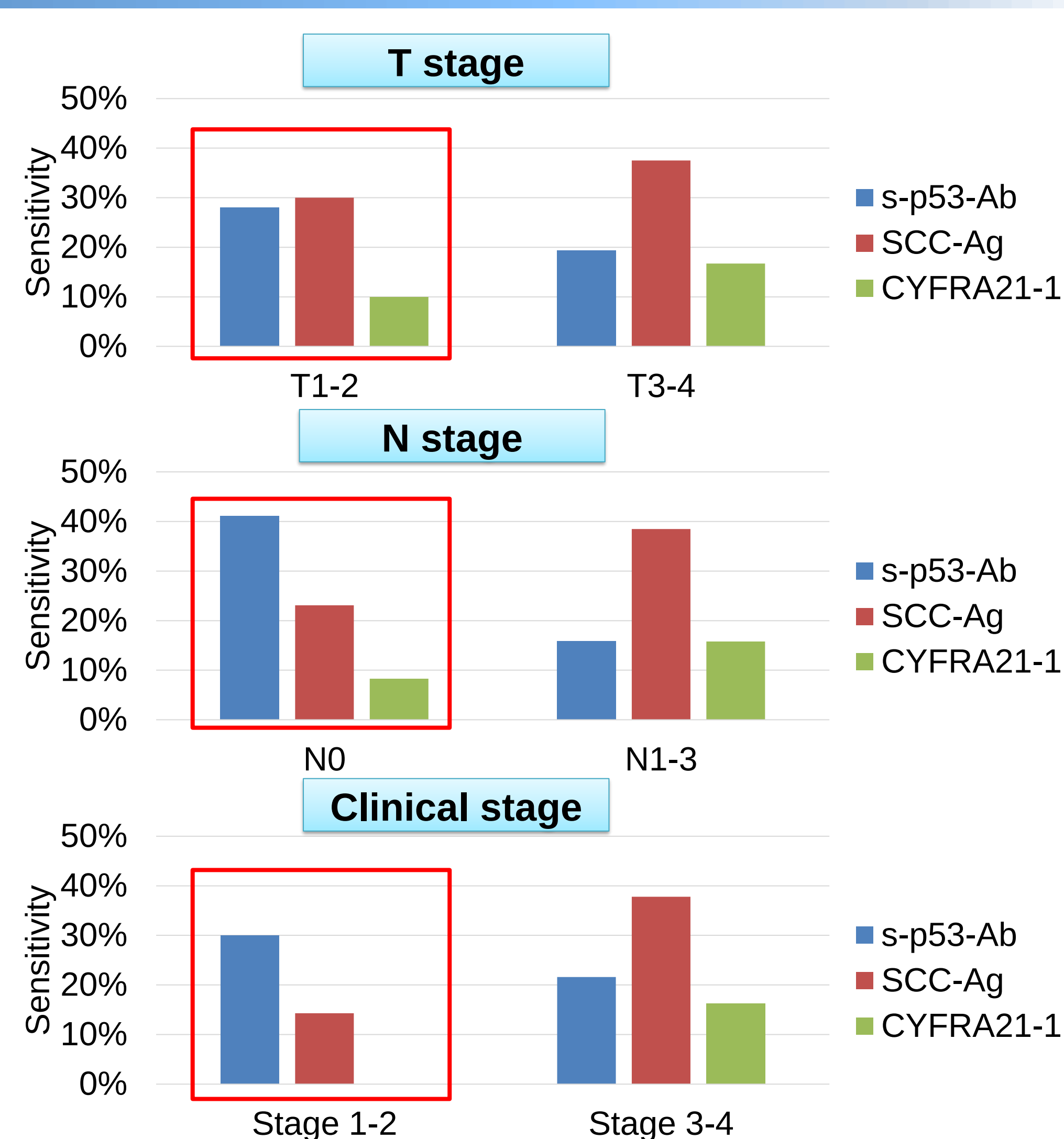


Figure 1. Sensitivity of biomarkers by disease progression in oral and non-virus-related pharyngeal cancer patients.

- The s-p53-Ab showed high sensitivity **even in early disease** with high specificity, compared to other conventional markers.

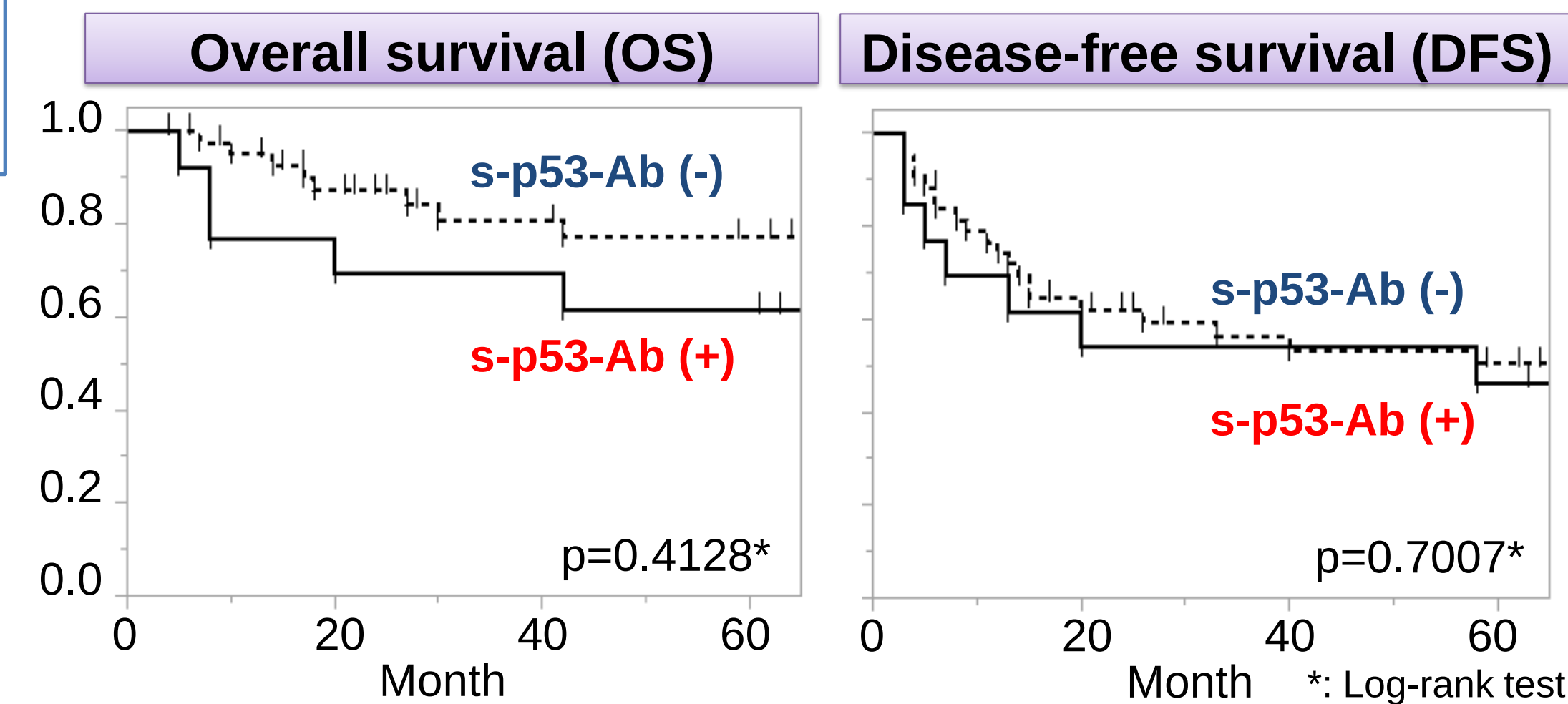


Figure 2. The correlation between prognosis and s-p53-Ab in oral and non-virus-related pharyngeal cancer patients.

- Fifty-six patients with oral and non-virus-related pharyngeal cancer who completed radical treatment
- Number of s-p53-Ab positive/negative: 13 / 43 patients
- There was **no statistically significant correlation between s-p53-Ab and prognosis** for both OS and DFS rate using Kaplan-Meier method.

Discussion

The positive rate of s-p53-Ab in patients with oral and pharyngeal cancer

– In previous reports for HNSCC patients: 16–24.7% [1-3]

– Our results: 19.7% (all patients)

✓ **Comparative, but high positive rate in non-virus-related cancer (23.0%)**

Classical oral / pharyngeal cancer (non-virus-related cancer)

- Associating with “**field cancerization**”[4]
- Frequently showing p53 gene mutation

↓
Accumulation of mutant p53 protein

↓
Induction of s-p53-Ab

Virus-related cancer

- Oncogenic mechanism is independent of p53 gene mutation

↓
Mutant p53 protein is not produced

↓
s-p53-Ab not induced

The value of s-p53-Ab as a biomarker in oral / pharyngeal cancer

For early diagnosis

- Useful for screening patients with non-virus-related cancer
 - Generally has a worse prognosis than virus-related cancer
 - High sensitivity and specificity even in early lesions** compared to SCC-Ag / CYFRA21-1
 - More useful in biomarker combinations**

→ **Contributes to early detection and early treatment**
Improving treatment outcomes

For prognosis prediction

- No significant significance was found in the association with prognosis

The limitation of the study and future issues

- Sample size of patients is small
 - Insufficient to examine the differences between primary sites in detail
- Large-scale multicenter joint research is required for future clinical application

Summary

- We measured serum p53 antibody in patients with oral and pharyngeal SCC and examined their value as novel clinical biomarkers.**
- The positive rate of s-p53-Ab was particularly high in patients with p16-negative oropharyngeal and hypopharyngeal cancers and was useful not only in advanced cancer but also in early cancer.**
- This study showed that s-p53-Ab can be used as a new biomarker for HNSCC treatment with relatively simple measurements and minimal patient burden.**

References

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