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# Core Fucosylation of Immunoglobulin G is a Biomarker Candidate for Monitoring Interstitial Pneumonia

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**Abstract**

Immunoglobulin G (IgG) carries *N*-glycans at the Asn 297 position of the CH2 domain, and modification of the glycan structure is a potential target for the diagnosis and monitoring of disease. To analyze *N*-glycan modification, we have developed a monoclonal antibody against IgG that carries a core fucose structure. Using this antibody, we used an enzyme-linked immunosorbent assay (ELISA) to measure the level of core fucosylated IgG in a patient's plasma. The patient underwent surgery for esophageal cancer and was then transferred to the intensive care unit due to respiratory insufficiency. A few days after admission, the patient was diagnosed with interstitial pneumonia. The level of core fucosylated IgG became lower (by approximately 40%) following the onset of interstitial pneumonia, and steroidal pulse therapy temporarily restored it. These results indicated that a low level of core fucosylated IgG would be useful for diagnosing interstitial pneumonia and could be used to monitor the disease state.

**Keywords:** Core Fucose; Immunoglobulin G (IgG); Interstitial Pneumonia; Biomarker; Case Report.

**Abbreviations:** IgG: immunoglobulin G; ICU: intensive care unit; ELISA: enzyme-linked immunosorbent assay; FUT8: α1,6-fucosyltransferase; CCL2: chemokine C-C motif ligand 2; CRP: C-reactive protein; SP-D: surfactant protein-D; PaO2: partial pressure of oxygen in arterial blood; PaCO2: arterial partial pressure of carbon dioxide.

## Introduction

Glycosylation is one of the most important post-translational modifications of proteins. Among the different types of glycosylation, *N*-glycosylation and O-glycosylation are essential for the regulation of glycoproteins that promote optimal functioning [1]. The glycan structure is well known as remarkably diverse and is often modified in pre-disease and disease states. For example, the glycan structures known as AFP-L3 [2] in primary hepatoma and CA19-9 (sialyl-Lewis A) [3] in gastrointestinal cancers are often used for the diagnosis and monitoring of these diseases.

The *N*-glycans of IgG are localized at the Asn297 amino acid position [4]. Agalactosylated IgG was found in early studies on rheumatoid arthritis, and reduced galactosylation of IgG glycans is now regarded as a sign of inflammation [5]. Moreover, recent studies have shown that the glycan structure of *N*-glycan in IgG

is altered also via sialylation and fucosylation in several diseases. For example, patients with ulcerative colitis show a reduction in the level of fucose structures in IgG [6]. On the other hand, patients with pancreatic cancer or renal cancer show an increased level of sialic acid structures, bisecting GlcNAc structures, and agalactose (non-galactosylation) structures in IgG [7,8]. A decrease in fucose structures in IgG in the early stages following infection with influenza virus has also been reported [9]. These reports strongly suggest that modification of the glycan structure in IgG would be a useful biomarker for diagnosing and monitoring some diseases. A lack of core fucose in IgG is well known to enhance antibody-dependent cellular cytotoxicity (ADCC) via increased FcgRIIIA binding and signalling [10,11].

In this study, we attempted to analyse the glycan structure of IgG, in particular the core fucose structure in *N*-glycans (Supplementary Figure S1), in a patient before and after the onset of interstitial pneumonia. For detection via ELISA, we used a monoclonal antibody against core fucosylated IgG that was recently developed in our laboratory [12].

## Materials and Methods

### Preparation of plasma samples

Surplus blood samples were collected into tubes that included an anti-coagulant. After gentle mixing by inversion, the tubes were centrifuged at 1,200 g for 10 min at room temperature and the supernatants served as plasma samples. The resultant samples were stored at -80°C.

### Enzyme-linked immunosorbent assay (ELISA)

The patient's plasma was diluted in PBS (1:20,000 or 1:40,000), and 100 µl of the diluted plasma was added to the wells of a 96-well plate (Sumitomo Bakelite Co., Ltd., Tokyo, Japan). After incubation overnight at 4 ℃, the wells were washed three times with 0.05% Tween20 in PBS. Then 100 µl of 5% (w/v) bovine serum albumin (BSA) (Sigma-Aldrich, Saint Louis, MO) in PBS was added and the mixture was incubated for 1 hr at RT for blocking. After washing the wells again three times with 0.05% Tween20 in PBS, 100 µl of an anti-core fucosylated human IgG antibody [1.25 µg/ml in 1% (w/v) of BSA in PBS] was added and the mixture was incubated for 2 hr at RT. The wells were then washed five times and incubated with an anti-mouse IgG antibody labelled with HRP (GE Healthcare, Chicago, IL) (1:2,000). After incubation for 1 hr at RT, the wells were washed again five times and the substrate solution [0.26 mg/ml of o-Phenylenediamine in 0.1 M citrate buffer (pH 5.0) including 0.009% H2O2] was added. After incubation for 30 min in the dark, the absorbance values at 492 nm were measured using an Infinite M Plex plate reader (TECAN, Männedorf, Switzerland). For detection of total IgG, an anti-human IgG antibody labelled with HRP (GE Healthcare) was used. For detection of SP-D, Human SP-D Quantikine ELISA Kit (R&D Systems, Minneapolis, MN) was used following the manufacturer's protocol.

### Case Presentation

The patient was in his 70s and had undergone surgery for esophageal cancer in our hospital and was then transferred to the intensive care unit (ICU) due to the occurrence of respiratory insufficiency (day 1). The patient's blood was collected in the ICU early each morning (usually around 5 – 6 am) and immediately prepared as plasma samples. Tracheal intubation was performed on day 5 and respiratory care was started using an artificial ventilator. On day 8, the patient was suspected of having interstitial pneumonia, and by day 9, CT analysis revealed ground-glass opacity in his right lung. Therefore, pulse therapy with a steroid (methylprednisolone sodium succinate) was performed on days 9 – 11. Following the pulse therapy, administration of another steroid (prednisolone) was started, and the patient's respiratory function was stabilized at a certain level (Supplementary Table S1). By day 18, CT analysis showed that the disease state of the patient's lung had worsened. During his stay in the ICU, the patient was administered other drugs including diuretics, psychotropics, and antibiotics. Infection with Pseudomonas aeruginosa was determined at certain time points via testing of the bacterial culture of the patient's sputum.

## Results

Core fucosylated IgG and total IgG in plasma samples were analysed by an enzyme-linked immunosorbent assay (ELISA). Both the amount of core fucosylated IgG and total IgG were decreased following diagnosis (days 11 – 14 and 17 – 24) (Figure 1A and 1B). The levels of core fucosylated IgG, which is the ratio of core fucosylated IgG relative to total IgG, were approximately 80% – 100% during days 1 – 10 but had decreased dramatically to approximately 40% by days 17 – 24 (Figure 2A). The steroid pulse treatment on days 9 – 11 resulted in temporary recovery of the level of core fucosylated IgG (days 15 and 16). The high level of acore fucosylated IgG (non-core fucosylated IgG) following the onset of interstitial pneumonia is shown in Figure 2B. The levels of C-reactive protein (CRP) and surfactant protein-D (SP-D) were also monitored during the patient's stay in the ICU and they are shown in Supplementary Figure S2.

Figure 1

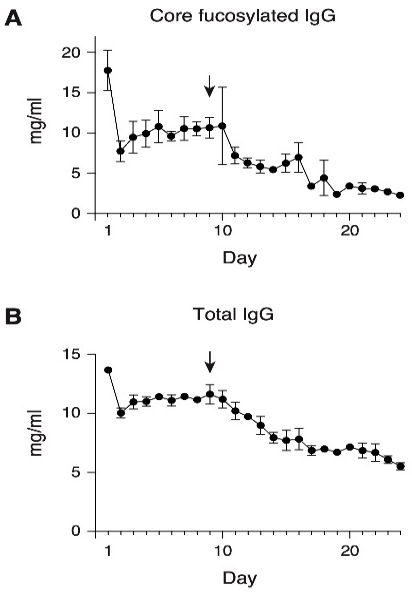



**Figure 1:** Levels of core fucosylated IgG and total IgG in the patient before and after the onset of interstitial pneumonia. The amounts of core fucosylated IgG (A) and total IgG (B) in plasma were measured by ELISA. Plasma samples were prepared and analyzed every other day. ELISA was performed in triplicate with values as means ± the standard deviation. The arrows indicate the day of the onset of interstitial pneumonia. Plasma samples were diluted in PBS at a ratio of 1:20,000 or 1:40,000 and analyzed in triplicate. The absorbance values were very low in the final detection step.

Figure 2

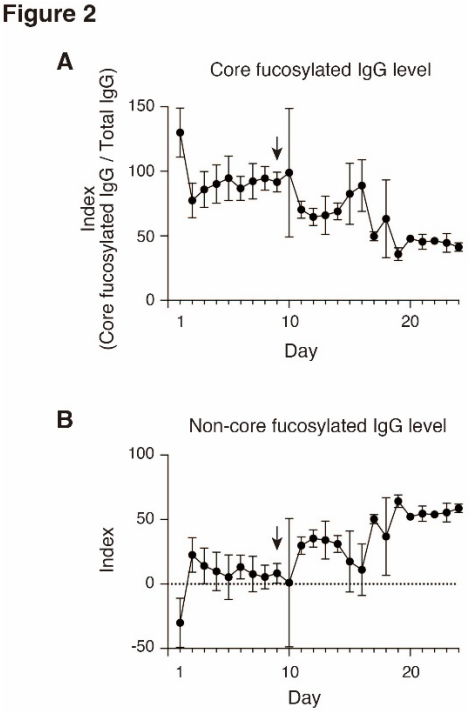
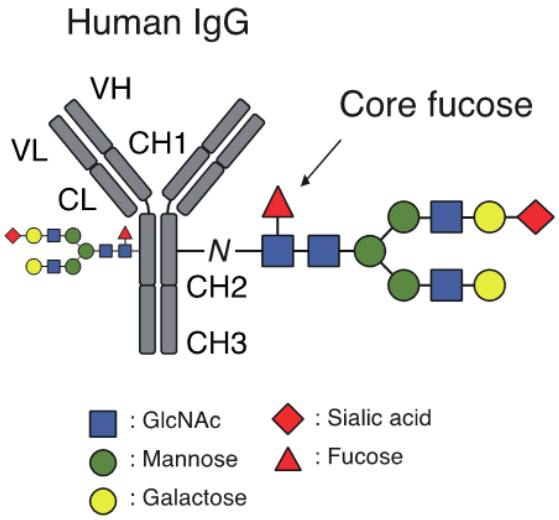


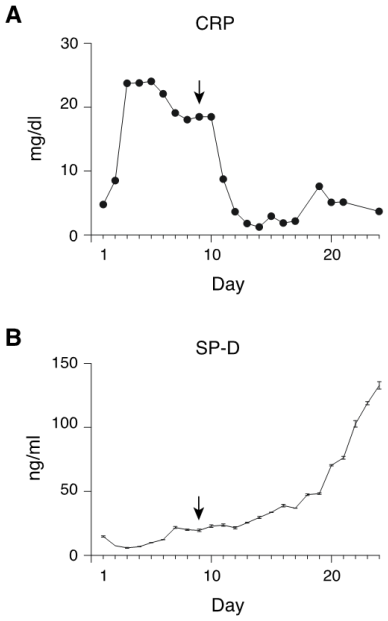

**Figure 2:** Levels of core fucosylated IgG and non-core fucosylated IgG relative to total IgG in the patient before and after the onset of interstitial pneumonia. The levels of core fucosylated IgG (A) and non-core fucosylated IgG (B) relative to total IgG in the plasma were calculated using the results shown in Figure 1. Values are reported as means ± the standard deviation. The arrows indicate the day of the onset of interstitial pneumonia.

# Supplementary Figure S1




**Supplementary Figure S1:** Core fucose structure in human immunoglobulin G (IgG). This figure is a modification of Figure 1A in our previously published paper [12].

## Supplementary Figure S2




**Supplementary Figure S2:** C-reactive protein (CRP) and surfactant protein-D (SP-D) in the patient's plasma. (A) The amount of CRP in the plasma was measured as a daily bedside routine. (B) The amount of SP-D in the plasma was measured via ELISA, which was performed in triplicate with values reported as the mean ± the standard deviation. The allows indicates the day of the onset of interstitial pneumonia.

| Day | $PaO_2$ (Torr) | $PaCO_2$ (Torr) | P/F ratio |
|---|---|---|---|
| 1 | - | - | - |
| 2 | - | - | - |
| 3 | - | - | - |
| 4 | 72 | - | - |
| 5 | 115 | 62 | - |
| 6 | - | - | - |
| 7 | 126 | - | 300 |
| 8 | - | - | - |
| 9 | 60 | - | Over 300 |
| 10 | 120 | 58 | - |
| 11 | 142 | 71 | - |
| 12 | 132 | 46 | - |
| 13 | - | - | - |
| 14 | 164 | 47 | Over 400 |
| 15 | - | - | 437 |
| 16 | - | - | - |
| 17 | - | - | - |
| 18 | - | - | - |
| 19 | 124 | 51 | - |
| 20 | - | - | 320 |
| 21 | - | - | 310 |
| 22 | - | - | 330 |
| 23 | 121 | 58 | 346 |
| 24 | 113 | 50 | 350 |

**Supplementary Table S1:** The patient's respiratory function during those days. Partial pressure of oxygen in arterial blood (PaO2), arterial partial pressure of carbon dioxide (PaCO2), and the P/F ratio are shown.

## Discussion

Many anti-cancer drugs such as those used in antibody therapies induce the onset of interstitial pneumonia as a side effect [13]. In addition to drug inducement, conditions such as smoking, bacterial infection, and autoimmune disease are some of the many reasons for the onset of interstitial pneumonia [14–16], and no radical treatment has yet been developed. This is why preventing the onset of interstitial pneumonia in cancer patients is an important issue.

The core fucose structure in N-glycans is biosynthesized via glycosyltransferase: α1,6-fucosyltransferase (FUT8). In our previous study, we found that chemokine C-C motif ligand 2 (CCL2) downregulated both the FUT8 gene and the level of core fucosylated IgG in IgG-secreting cells. We determined that the inflammatory cytokine interleukin-6 (IL-6) could also have been involved in the downregulation of the FUT8 gene [12]. These results indicated that the inflammation induced by inflammatory cytokines and chemokines was involved in the decrease of core fucosylated IgG levels. The results of previous studies combined with the results of the present study show that the inflammatory status of a patient with interstitial pneumonia can be monitored via the level of core fucosylated IgG. Decreases in core fucosylated IgG in COVID-19 patients have recently been reported [17–19]. Low levels of core fucosylated IgG have also been found in patients with thyroiditis and autoimmune diseases of the thyroid [20]. These findings also suggest a possible benefit of using core fucosylated IgG levels to monitor the inflammatory status.

C-reactive protein (CRP) is a good index for monitoring the inflammatory status of patients, and surfactant protein-D (SP-D) is a well-known clinical biomarker of interstitial pneumonia [21]. In the present case, CRP levels in the patient were dramatically reduced by steroid pulse treatment, while the core fucosylated IgG levels were persistently low during days 12 – 14. These results and the fact that the patient's condition had worsened again after day 18 suggested that core fucosylated IgG levels could also be another useful index for monitoring the disease state of interstitial pneumonia. On the other hand, SP-D levels were dramatically increased after day 20. Notably, the diagnostic baseline of SP-D is 110 ng/ml, and, hence, by following SP-D levels, the patient was diagnosed with interstitial pneumonia on day 23. These results strongly suggest that core fucosylated IgG is a more predictive biomarker than SP-D.

## Conclusions

This report is the first report to suggest the possibility that establishing low levels of core fucosylated IgG, in other words, a high level of a core fucosylated IgG (non-core fucosylated IgG), could be useful for the diagnosis of interstitial pneumonia and for monitoring the disease state of this condition.

Figures and Figure legends

## Declarations

**Availability of data and material:** All data generated or analysed during this study are included in this published article and its supplementary information files.

**Consent for publication:** All authors agreed to the submission and publication of this manuscript.

**Ethics approval and consent to partici**pate: All studies using the patient's samples were approved by the local ethics committee at Osaka International Cancer Institute (Approval number 21099-2). Informed consent was obtained prior to sample collection, and the patient agreed that his samples would be analyzed for research purposes only and that the results would be published.

**Competing interests:** The authors declare no conflicts of interest that could appear to influence the results of this study.

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**Authors' contributions:** YOhk, HT, and NT designed the study. YOha collected the plasma samples. YOhk, RF, and KN performed the experiments. YOhk and RF analyzed the data. YOhk drafted the manuscript. YH, HT, and NT revised the manuscript.

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