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**Original Article**

# Elevation of soluble Fas (APO-1, CD95) ligand in natural aging and Werner syndrome

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**Summary**

The pathophysiological process of natural human aging has not been studied adequately due to the lack of an appropriate human model. Since recent investigations have suggested that inflammation possibly contributes to the pathogenesis of age-related disorders including atherosclerosis, cancer, and diabetes mellitus, the term "inflammaging," a combination of "inflammation" and "aging," has been coined. Werner syndrome (WS), caused by the loss of function of RecQ3 DNA/RNA helicase, is a typical progeroid syndrome mimicking natural aging, although it is extremely rare outside of Japan. We sought to examine WS patients from an immunological/inflammatory perspective. Sera from 14 mutation-proven WS patients (ages: 33-70 years) and 21 healthy Japanese adults ages 15 to 95 years were examined with ELISA for soluble Fas ligand (sFasL) to compare conventional inflammation markers. With natural aging, a statistically significant correlation ( $p < 0.0001$ ) was observed in the serum level of sFasL. The sFasL in WS, a level comparable to that in healthy elderly ages 83 to 95 years, had significantly increased ( $p < 0.05$ ) compared to that in young healthy individuals ages 15 to 70 years. A significant correlation was noted between the serum levels of conventional inflammation markers such as CRP ( $p < 0.025$ ), ESR ( $p < 0.024$ ), and WBC count ( $p < 0.0085$ ). In conclusion, an increased level of serum sFasL in natural aging and WS patients may suggest a common pathophysiological mechanism: inflammation. WS may be a good model for analyzing inflammaging.

**Keywords:** Aging, Apoptosis, Inflammaging, Soluble Fas ligand, Werner syndrome

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## 1. Introduction

Apoptosis, known as programmed cell death, is reported to be essential as a silent, chronic physiological process necessary for part of normal development and organ homeostasis through the interaction of death factors (TNF, FasL, and TRAIL) and cell surface death receptors (Fas, TNFR1, DR3/Wsl-1, and CAR1); thus, it differs from another type of dynamic cell death accompanied by acute inflammation known as necrosis (1-5). FasL is a 40-kd type II transmembrane protein that belongs to the TNF family (5); it induces apoptosis

through its membrane receptor, Fas. FasL is converted to the 26-kd soluble form of the truncated membrane-bound Fas receptor known as the sFas ligand (sFasL) by inflammatory metalloproteinases (6).

Recent investigations have suggested the critical role of apoptosis in the development of a variety of aging-related chronic diseases known as apoptotic syndromes such as Alzheimer's disease, systemic lupus erythematosus, and osteoporosis (7). Because of mounting studies on chronic inflammation, apoptosis is also believed to be the mechanism for the resolution of acute inflammation through the phagocytosis of neutrophils and other cells by macrophages at the site of inflammation (8), clearing up the apoptotic cells at an earlier stage without inciting secondary inflammation due to nitric oxide (9). However, if such a process goes wrong, then apoptosis proceeds to a later stage, following by the possible induction of silent chronic

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inflammation by producing pro-inflammatory cytokines (10).

Aging has been defined as a biologically declining process leading to organismal death at the latter stage of our life in contrast to development, growth, and maturation. Although several researchers have suggested that apoptosis has a role in aging, there has been no concrete evidence indicating its direct role in natural aging (11-13).

Although the pathophysiological functions of sFasL have not been fully clarified, sFasL inhibits Fas-mediated apoptosis by competitively binding with FasL and altering lymphocyte development and proliferation in response to self-antigens (14,15). Elevation of sFasL in the circulation has also been detected in autoimmune rheumatic diseases (14-17), atherosclerosis (18,19), obesity (20), and malignancies (21,22). The relationship between the serum level of sFasL and chronic inflammation has been a matter of considerable discussion especially in the context of the natural aging process (20-24).

Werner syndrome (WS; MIM#27770), a typical progeroid syndrome, has been nominated as the best natural model for analyzing human aging (25-27), as the syndrome manifests a variety of age-related signs and symptoms including skin atrophy, skin pigmentation, sarcopenia, cataracts, diabetes mellitus, hypogonadism, osteoporosis, atherosclerosis, and malignancy at a relatively early stage of life followed by an early death (28,29). Interestingly, a majority of patients with this illness are Japanese in origin, partly because of the relatively high prevalence of consanguineous marriage in rural areas and the extremely high frequency of heterozygotes in Japan (30,31). The current authors previously reported inflammatory conditions observed in WS in a series of publications (32-38). Here, the intent was to study the pathophysiological roles of serum sFasL in the development of aging-related signs and symptoms in Japanese patients with WS and healthy individuals.

## 2. Materials and Methods

### 2.1. Study population

Serum samples were obtained from 14 patients with mutation-proven WS (M = 6, F = 8; ages 33 to 70 years) (28-31,38), 13 healthy young volunteers (M = 4, F = 9; ages 15 to 70 years), and 8 healthy elderly volunteers (M = 1, F = 7; ages 83 to 95 years) who provided informed consent for this study, which was approved by the ethics committee of Tooin University of Yokohama. All samples were stored at -80°C until use. All of the WS patients showed the following manifestations as previously reported: typical body status/face, hoarseness, gray hair/alopecia, skin atrophy/sclerosis/pigmentation, sarcopenia, cataracts, osteoporosis, and subcutaneous calcification. As indicated in Table 1, 11

**Table 1.** Clinical characteristics in Werner syndrome patients

Age	Sex	DM	Hyper-lipidemia	Skin ulcer	Hyper-uricemia	Malignancy pre/post
39	F	+	+	-	-	-/+
39	M	+	+	-	+	-/-
40	F	+	+	+	-	+/-
42	M	-	-	-	+	-/-
45	F	+	-	+	+	-/-
47	M	-	+	-	-	-/+
50	F	+	-	+	+	-/-
50	F	-	+	+	+	-/+
52	F	+	+	+	-	-/-
52	F	-	+	-	+	-/+
54	M	+	+	+	-	-/-
55	M	+	-	-	-	-/-
59	F	-	+	+	+	-/-
70	M	+	+	+	+	-/-

In the malignancy column, -/+ indicates patients did not have a malignancy before sampling but had one after sampling. +/- indicates a patient had a malignancy before sampling but did not have one after sampling.

patients had hyperlipidemia, 9 had diabetes mellitus, 8 had skin ulcers, and 7 had hyperuricemia. No patient had a malignancy at the time of sampling, but one had a malignancy before sampling and 4 had one after sampling (28-30,40).

### 2.2. Determination of sFasL and inflammation markers

sFasL was measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions (MBL, Nagoya, Japan) (41). The ESR level (mm/h) was measured by the Westergren method. CRP (ng/mL) was measured by nephelometry. The number of WBCs was counted by the standard method.

### 2.3. Statistical analysis

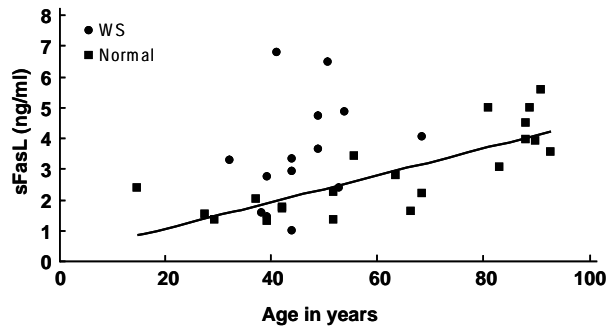
Data are expressed as mean  $\pm$  SE. Significance was tested using the unpaired *t*-test, while correlations were determined using Pearson's formula.

## 3. Results and Discussion

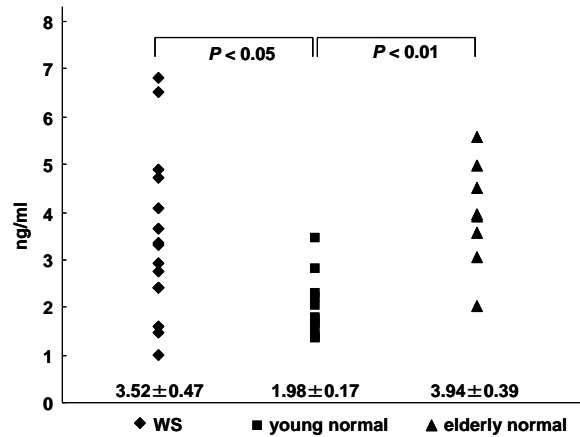
The serum level of sFasL was significantly correlated with natural aging ( $r = 0.8415$ ,  $p < 0.0001$ ) (Figure 1) but there was no gender difference as was previously reported (20,23,42) (Figure 1).

The sFasL level in WS patients was significantly higher ( $3.52 \pm 0.47$  ng/mL;  $p < 0.05$ ) than that in age- and sex-matched young healthy individuals ( $1.98 \pm 0.17$  ng/mL), but was comparable to that in elderly healthy individuals ( $3.94 \pm 0.39$  ng/mL), as shown in Figure 2.

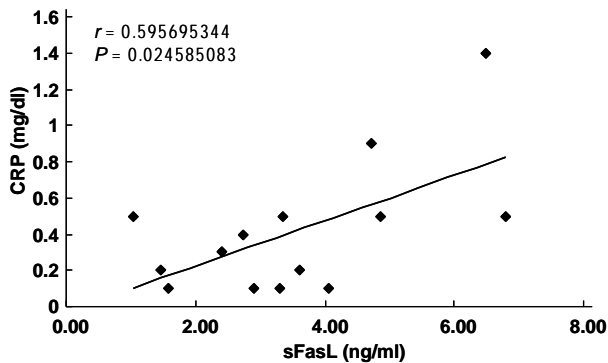
sFasL may be produced as a result of inflammation, and significant correlations were observed between serum levels of sFasL and inflammation markers, *i.e.* CRP ( $r = 0.596$ ;  $p < 0.025$ ) (Figure 3), ESR ( $r = 0.598$ ;  $p < 0.024$ ) (Figure 4), and WBC counts ( $r = 0.67$ ;  $p < 0.0085$ ), in WS patients; these correlations have been described in the general population as well



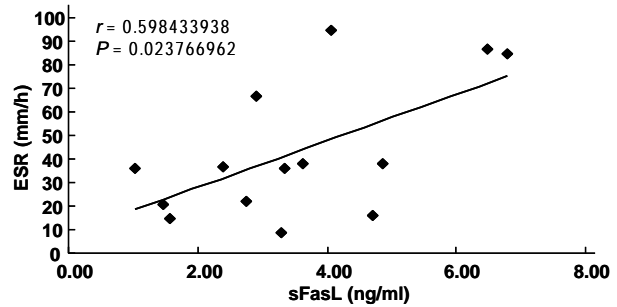
**Figure 1.** sFasL in Werner syndrome and natural aging. Serum levels of sFasL: sFasL were assayed with a commercial sFasL ELISA kit as described in the Materials and Methods section. A statistically significant correlation ( $p < 0.0001$ ) was observed between the serum level of sFasL and natural aging. The sFasL level in most WS patients increased in comparison to that in age-matched healthy individuals.



**Figure 2.** The serum sFasL significantly increased ( $p < 0.05$ ) in comparison to that in age-matched healthy young individuals but was comparable to that in elderly individuals. The mean  $\pm$  SE of the serum sFasL level in each group is indicated in the figure.



**Figure 3.** Correlation between serum levels of sFasL and CRP. The serum level of sFasL was significantly associated with an increasing level of CRP ( $p < 0.025$ ).



**Figure 4.** Correlation between serum levels of sFasL and ESR. The serum level of sFasL was significantly associated with an increasing level of ESR ( $p < 0.024$ ).

(23,41,42). Patients with WS manifest metabolic syndrome consisting of hyperlipidemia, atherosclerosis, diabetes mellitus and hyperuricemia, skin ulcers, and malignancy (28-30,39), all of which have been shown to be closely linked to chronic inflammation (23,37,43,44). Partly because of the limitations of the samples studied, however, statistically significant differences in serum levels of sFas, CRP, ESR, and WBC were not detected between individuals with and without DM, hyperlipidemia, skin ulcers, hyperuricemia, and malignancy.

Although the exact role of serum sFasL in aging has not been clarified, apoptosis in development and maturation may be a prologue to the subsequent chapter of chronic inflammation in senescence, followed by organismal death. Thus, WS may be a good model for studying how low-grade inflammation leads to senescence ('inflammaging') (45).

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