なし

2. 実用新案登録
なし

3. その他
なし

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書籍

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Charvat H, Sasazuki S, Inoue M, Iwasaki M, Sawada N, Shimazu T, Yamaji T, Tsugane S; for the JPHC Study Group.	Prediction of the 10-year probability of gastric cancer occurrence in the Japanese population: the JPHC Study Cohort II.	Int J Cancer	138 (2)	320-31	2016
Cai H, Ye F, Michel A, Murphy G, Sasazuki S, Taylor PR, Qiao Y-L, Park SK, Yoo K-Y, Jee SH, Cho ER, Kim J, Kim Chen S-C, Abnet CC, Tsugane S, Cai Q, Xiao-Ou Shu, Zheng W, Pawlita M Epplein M.	Helicobacter pylori blood biomarker for gastric cancer risk in East Asia	Int J Epidemiol			2016 in press
Hidaka A, Sasazuki S, Matsuo K, Ito H, Hadrien C, Sawada N, Shimazu T, Yamaji T, Inoue M, Iwasaki M, Tsugane S; for the JPHC Study Group.	CYP1A1, GSTM1, and GSTT1 genetic polymorphisms and gastric cancer risk among Japanese: A nested case-control study within a large-scale population-based prospective study	Int J Cancer			2016 in press
Ma E, Sasazuki S, Shimazu T, Sawada N, Yamaji T, Iwasaki M, Inoue M, Tsugane S.	Reactive oxygen species and gastric cancer risk: a large nested case-control study in Japan.	Eur J Epidemiol	30 (7)	589-94	2015

Katanoda K, Kamo KI, Tsugane S.	Quantification of the increase in thyroid cancer prevalence in Fukushima after the nuclear disaster in 2011-a potential overdiagnosis?	Jpn J Clin Oncol	46 (3)	284-286	2016
Hamashima C	Have we comprehensively evaluated the effectiveness of endoscopic screening for gastric cancer?	Asian Pacific Journal of Cancer Prevention.	16 (8)	3591-3592	2015
Hamashima C, Shabana M, Okamoto M, Osaki Y, Kishimoto T	Survival analysis of patients with interval cancer undergoing gastric cancer screening by endoscopy.	PLoS ONE	10(5)	e0126796	2015
Lauby-Secretan B, Scoccianti C, Loomis D, Benbrahim-Tallaa L, Bouvard V, Bianchini F, Straif K, for the International Agency for Research on Cancer Handbook Working Group: [Armstrong B, Anttila A, de Koning HJ, Smith RA, Thomas DB, Weiderpass E, Anderson BO, Badwe RA, da Silva TCF, de Bock GH, Duffy SW, Ellis I, Hamashima C, Houssami N, Kristensen V, Miller AB, Murillo R, Paci E, Patnick J, Qiao YL, Rogel A, Segnan N, Shastri SS, Solbjor M, Heyyang-Kobrunner SH, Yaffe MJ, Forman D, von Karsa Lawrence, Sankaranarayanan R	Breast-Cancer Screening — Viewpoint of the IARC Working Group.	The New England Journal of Medicine	372(24)	2353-2358	2015

Hamashima C, Ohta K, Kasahara Y, Katayama T, Nakayama T, Honjo S, Ohnuki K	A meta-analysis of mammographic screening with and without clinical breast examination.	Cancer Sci.	106(7)	812-8	2015
Hamashima C	The Japanese guideline for breast cancer screening.	Jpn J Clin Oncol.			2016 in press
Tanaka S, Saitoh Y, Matsuda T, Igarashi M, Matsumoto T, Iwao Y, Suzuki Y, Nishida H, Watanabe T, Sugai T, Sugihara K, Tsuruta O, Hirata I, Hiwatashi N, Saito H, Watanabe M, Sugano K, Shimosegawa T.	Evidence-based clinical practice guidelines for management of colorectal polyps.	The Japanese Society of Gastroenterology	50 (3)	252-60	2015
Ohuchi N, Suzuki A, Sobue T, Kawai M, Yamamoto S, Zheng Y, F, Narikawa Shiono Y, Saito H, Kuriyama S, Tohno E, Endo T, Fukao A, Tsuji I, Yamaguchi T, Ohashi Y, Fukuda M, Ishida T, for the J-START investigator groups.	Sensitivity and specificity of mammography and adjunctive ultrasonography to screen for breast cancer in the Japan Strategic Anti-cancer Randomized Trial (J-START): a randomised controlled trial.	Lancet	387(10016)	341-8	2016
Taniguchi T, Hirai K, Harada K, Ishikawa Y, Nagatsuka M, Fukuyoshi J, Arai H, Mizota Y, Yamamoto S, Saito H, & Shibuya D.	The relationship between obtaining fecal occult blood test and beliefs regarding testing among Japanese.	Health Psychology and Behavioral Medicine	3(1)	251-262	2015

Sekiguchi M, Igarashi A, Matsuda T, Matsumoto M, Sakamoto T, Nakajima T, Kakugawa Y, Yamamoto S, Saito H, Saito Y.	Optimal use of colonoscopy and fecal immunochemical test for population-based colorectal cancer screening: a cost-effectiveness analysis using Japanese data.	Jpn J Clin Oncol	46 (2)	116-25.	2016
Young GP, Senore C, Mandel JS, Allison JE, Atkin WS, Benamouzig R, Bossuyt PM, Silva M, Guittet L, Halloran SP, Haug U, Hoff G, Itzkowitz SH, Leja M, Levin B, Meijer GA, O'Morain CA, Parry S, Rabeneck L, Rozen P, Saito H, Schoen RE, Seaman HE, Steele RJ, Sung JJ, Winawer SJ.	Recommendations for a step-wise comparative approach to the evaluation of new screening tests for colorectal cancer.	Cancer	122 (6)	826-39	2016
斎藤　博.	大腸がん検診のあり方―最近のエビデンスを踏まえて	診療と治療	103(2)	173-178	2015
奥山絢子、東　尚弘、斎藤　博、雑賀公美子、町井涼子、松田和子、若尾文彦.	がんの早期発見分野におけるがん対策進捗管理指標と進捗状況	癌の臨床	61(2)	155-161	2015
斎藤　博.	がん対策としての大腸がん検診の現状と問題点	消化器内視鏡	27(4)	589-594	2015
斎藤　博、高橋則晃、町井涼子.	検診で死亡率を下げる方策を探る	医学のあゆみ	253(10)	977-983	2015
斎藤　博、雑賀公美子、町井涼子、高橋則晃.	産婦人科必読　乳がん予防と検診Up to date【乳がんの疫学と予防】検診による死亡率低下の重要性	臨床婦人科産科	69 (6)	498-503	2015

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Prediction of the 10-year probability of gastric cancer occurrence in the Japanese population: the JPHC study cohort II

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Cancer Epidemiology

Gastric cancer is a particularly important issue in Japan, where incidence rates are among the highest observed. In this work, we provide a risk prediction model allowing the estimation of the 10-year cumulative probability of gastric cancer occurrence. The study population consisted of 19,028 individuals from the Japanese Public Health Center cohort II who were followed-up from 1993 to 2009. A parametric survival model was used to assess the impact on the probability of gastric cancer of clinical and lifestyle-related risk factors in combination with serum anti-*Helicobacter pylori* antibody titres and pepsinogen I and pepsinogen II levels. Based on the resulting model, cumulative probability estimates were calculated and a simple risk scoring system was developed. A total of 412 cases of gastric cancer occurred during 270,854 person-years of follow-up. The final model included (besides the biological markers) age, gender, smoking status, family history of gastric cancer and consumption of highly salted food. The developed prediction model showed good predictive performance in terms of discrimination (optimism-corrected *c*-index: 0.768) and calibration (Nam and d'Agostino's χ^2 test: 14.78; *p* values = 0.06). Estimates of the 10-year probability of gastric cancer occurrence ranged from 0.04% (0.02, 0.1) to 14.87% (8.96, 24.14) for men and from 0.03% (0.02, 0.07) to 4.91% (2.71, 8.81) for women. In conclusion, we developed a risk prediction model for gastric cancer that combines clinical and biological markers. It might prompt individuals to modify their lifestyle habits, attend regular check-up visits or participate in screening programmes.

Key words: gastric cancer, *Helicobacter pylori*, pepsinogen, prediction model
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Stomach cancer is currently the fifth most common cancer, with approximately 1 million new cases diagnosed worldwide each year, and ranks as the third leading cause of cancer-related death.[1] It represents a particularly important public health issue in East Asian countries, where incidence rates are among the highest observed. In Japan, although the age-standardized incidence rate has been continually decreasing for a few decades, the number of new cases still exhibits an increasing pattern that is thought to be mainly attributable to the ageing of the population.[2] According to recently published national cancer statistics,[3] gastric cancer was the most commonly diagnosed cancer, accounting for 15.6% of cancer cases diagnosed in 2010, and still represented the second most important cause of cancer-related death in 2012.

Gastric carcinogenesis is a well-studied process thought to be mainly related to chronic infection with *Helicobacter pylori*,[4] resulting in atrophic gastritis, intestinal dysplasia, metaplasia and, finally, adenocarcinoma.[5] A recent update of the worldwide global burden of gastric cancer related to infections[6] estimated that almost 90% of noncardia gastric cancers were attributable to *H. pylori*.[7] It is thought that *H. pylori* might be almost a necessary condition for gastric cancer development and that negativity of *H. pylori* infection in gastric cancer cases reflects the inability of the bacteria to develop on the mucosa of patients with severe atrophic

What's new?

Gastric cancer is still one of the most common and deadly cancers, especially in Japan. In this study, the authors developed a model and a simple scoring system to estimate an individual's risk of developing gastric cancer, based on factors such as *H. pylori* antibodies, serum pepsinogen levels, and lifestyle habits. This predictive model may encourage people to modify their habits or participate in screening programmes, and may also enhance our understanding of *H. pylori* infection and atrophic gastritis as contributing factors in long-term gastric cancer risk.

gastritis.[8–10] Nonetheless, *H. pylori* is not a sufficient cause of gastric cancer, and other risk factors that may modulate the impact of the infection, such as cigarette smoking[11–13] and dietary habits (highly salted food consumption,[14] intake of fruit and vegetables[15,16]), have been identified.[17]

Because the symptoms associated with the development of gastric cancer are nonspecific, diagnosis is often made at an advanced stage of the disease, resulting in a poor survival rate.[5] However, the situation is slightly different in Japan, where the 5-year relative survival rate is more than two times higher than that observed in other countries; this difference is considered to be mainly attributable to early-stage diagnosis.[2] From the standpoint of prevention, gastric cancer thus represents a particularly important target.

Several risk prediction models for gastric cancer have already been developed. For example, Buckland *et al.* developed a prognostic model based on a large cohort population to assess the impact of lifestyle factors on the risk of occurrence of gastric cancer;[18] Lee *et al.* proposed a prognostic tool aimed at predicting cumulative incidence of gastric cancer in patients with peptic ulcer,[19] while Tata *et al.* developed a diagnostic scoring system based on clinical variables.[20] However, these models do not take into account information on the presence of *H. pylori* infection and atrophic gastritis, which are strong predictors of the risk of gastric cancer occurrence, especially in the Japanese population.[9] Miki proposed a test for risk stratification based on anti-*H. pylori* IgG antibody and serum pepsinogen levels called the "ABC method,"[9] which allows the classification of individuals in four categories of increasing risk. Several studies have demonstrated its usefulness in discriminating high-risk individuals who may benefit from further gastric cancer screening procedures.[10,21,22] These studies are nonetheless of relatively small size and do not consider the concomitant effect of lifestyle risk factors.

A screening programme based on an X-ray method with photofluorography is currently used in Japan.[23] However, applying this screening method to all Japanese individuals after a certain age would not be cost-effective.[21] On the other hand, informing individuals on their risk of gastric cancer is an important aspect of prevention.[24] In that respect, the recent development of the "ABC method"[9] constitutes a promising tool. Besides, risk stratification might be further enhanced by taking into account risk factors known to impact on gastric cancer occurrence, such as smoking habits or salt consumption. Therefore, there is a need for the development of a simple tool that allows the estimation of the risk of gastric cancer occurrence based on simple clinical, biological and lifestyle-related factors. It might help clinicians to persuade their patients to modify their lifestyle habits, attend regular check-up visits or participate in more specific screening programmes.

The aim of this work was thus to improve on existing studies by constructing a prognostic risk prediction model and a simple scoring system allowing the estimation of the 10-year cumulative probability of gastric cancer occurrence based on a large cohort population with information on easily obtainable clinical and lifestyle-related characteristics in combination with serum measurements of anti-*H. pylori* IgG antibodies and levels of pepsinogen I and pepsinogen II.

Material and Methods

Study population

The participants in our study were Japanese residents included in the Japanese Public Health Center (JPHC)-based Prospective Study Cohort II[25] recruited during the period 1993–1994 who answered a self-administered baseline questionnaire distributed at study entry and provided a blood sample collected at the same time or during the year after completion of the questionnaire (mean time between questionnaire and blood sample collection: 0.44 years). The starting point was then defined for each subject as the date of completion of the baseline questionnaire or the date of blood sample collection, whichever came last, and age at the start of the study was recalculated accordingly. Subjects were followed-up until 31 December 2009. Supporting Information Figure S1 describes the study population selection process. The Institutional Review Board of the National Cancer Center, Tokyo, Japan, approved the study.

Follow-up and identification of gastric cancer cases

Residency registration and death registration are required by the Basic Residential Register Law and Family Registry Law, respectively, and the registries are thought to be complete. We identified incident gastric cancer cases by active patient notification from major local hospitals in each study area and from data linkage with population-based cancer registries. Cancer cases were coded according to the International Classification of Diseases for Oncology, 3rd edition,[26] gastric cancer corresponding to codes C-160 to C-169.

Laboratory analyses

Serum levels of IgG antibodies to *H. pylori* were measured by enzyme immunoassay (E plate "Eiken" *H. pylori* Antibody II; Eiken Kagaku, Tokyo, Japan). Seropositivity for anti-*H. pylori* antibodies was defined as an IgG titre $\geq$ 10 U/mL. Serum levels of pepsinogen I and pepsinogen II were measured by latex agglutination (LZ test "Eiken" Pepsinogen I, II; Eiken Kagaku). "Atrophic gastritis" was defined when the criteria of both a pepsinogen I level $\leq$70 ng/mL and a pepsinogen I/II ratio $\leq$3.0 were fulfilled. The four categories of the "ABC method"[9] were then defined as follows: category A represented individuals negative for both anti-*H. pylori* IgG and atrophic gastritis; category B represented individuals positive for only anti-*H. pylori* IgG; category C represented individuals positive for both anti-*H. pylori* IgG and atrophic gastritis and category D represented individuals positive only for atrophic gastritis.

Preliminary selection of risk factors

In the following, we list the potential risk factors that were considered in preliminary analyses, and explain, for each of them, the reasons for selecting them or not in the subsequent model building procedure.

Smoking status. Smoking has been identified as a risk factor for gastric cancer.[11–13] In our work, preliminary analyses showed that past smokers had the same risk as never smokers, so smoking status was defined as a dichotomous variable in the final analysis (never and past smokers *vs.* current smokers).

Alcohol consumption. Although the role of alcohol in the development of gastric cancer has long been debated, a recent meta-analysis showed that heavy drinking was significantly associated with non-cardia gastric cancer.[27] However, the analysis restricted to Asian studies suggested an absence of excess risk, because the aldehyde dehydrogenase and alcohol dehydrogenase polymorphisms present in Asian populations might somewhat limit alcohol consumption levels. Preliminary analyses were also consistent with the absence of effect of alcohol in our study population, so we did not consider it further in our model building procedure.

Salt and highly salted food consumption. Total salt and salted food consumption have been classified as "probable" risk factors for gastric cancer occurrence in the second expert report on diet and cancer of the World Cancer Research Fund.[7] In a cohort study in the Japanese population, Tsugane *et al.*[14] found an association between the consumption of various highly salted food items and the risk of gastric cancer. In our study, we assessed in preliminary analyses the effect of various salted food items (pickled green vegetables, other pickled vegetables and salted fish roe) as well as the estimated total salt consumption. Only salted fish roe consumption was associated with an increased risk of gastric cancer. Salted fish roe is not part of the everyday diet and the distribution of the frequency of salted fish roe consumption was highly skewed with almost 45% of individuals never consuming it and 45% eating it less than once a week. We thus decided to dichotomize salted cod roe consumption into never *vs.* at least sometimes in subsequent analyses. More than the actual consumption of salted cod roe, we believe that this variable indicates an inclination for highly salted food in general.

Fruit and vegetable consumption. Fruit and vegetable have previously been found to be associated with gastric cancer.[15,16,28] In particular, fruits, nonstarchy and allium vegetables were classified as "probable" risk factors for gastric cancer in the second expert report on diet and cancer of the World Cancer Research Fund.[21] However, preliminary analyses in our study population failed to find any effect of fruit and vegetable consumption. Moreover, the questionnaire used in the JPHC Study Cohort II does not allow us to isolate nonstarchy or allium vegetable consumption. Finally, our aim was to develop a risk prediction model that should be easy to use and therefore could not be based on detailed questionnaires assessing food consumption. Consequently, we did not consider fruit and vegetable consumption in the model building procedure.

Family history and hereditary cancer syndromes. Family history of gastric cancer has been described as a risk factor for gastric cancer. However, it appears that known hereditary cancer syndromes [such as Lynch syndrome, familial adenomatous polyposis (FAP) and Peutz-Jeghers syndrome] account for only a small proportion of familial gastric cancer cases aggregations and it is thought that familial clustering is mainly related to shared environmental exposures and genetic susceptibilities associated with low-penetrance alleles.[29] In our study, we defined family history as a history of gastric cancer for at least one first-degree relative (parents and siblings).

Statistical analysis

Person-years of follow-up for each individual were calculated from the starting point to the date of gastric cancer diagnosis, date of emigration from the study area, date of death or end of follow-up (31 December 2009), whichever came first.

Semi-parametric Cox proportional hazards models were used for covariate selection. Variables corresponding to already known and validated risk factors (*i.e.,* age, gender, the categories defined by the combined anti-*H. pylori* IgG antibody and serum pepsinogen status) were introduced in the model without prior selection step. In addition, we considered three factors that have been found to be associated with gastric cancer: smoking status,[11–13] consumption of highly salted food[14] and family history of gastric cancer.[29] The various combinations of one or more of these factors were added in turn to the basal linear predictor and selection of the appropriate combination was performed by comparing the Akaike information criteria (AIC) of the different models obtained. Statistical interaction between age and the selected

Table 1. Baseline demographic and clinical characteristics of the study population consisting of 19,028 individuals from the JPHC Cohort II

	Individuals free of gastric cancer		Gastric cancer cases	
Baseline characteristics	$n = 18,616$	Proportion (%)	$n = 412$	Proportion (%)
Age	59.3 (51.5, 64.9)[1]		63.3 (57.9, 67.1)[1]	
Gender				
Women	11,979	64.3	157[2]	38.1
Men	6,637	35.7	255	61.9
Smoking status				
Never	13,161	70.7	199	48.3
Past	2,128	11.4	82	19.9
Current	3,327	17.9	131	31.8
Family history (parents or siblings)				
No	17,390	93.4	373	90.5
Yes	1,226	6.6	39	9.5
Fish roe, fish gut consumption				
No	8,275	44.5	150	36.4
Yes	10,341	55.5	262	63.6
Atrophic gastritis[2]				
No	10,900	58.6	116	28.2
Yes	7,716	41.4	296	71.8
Anti-H. pylori IgG seropositivity[3]				
No	5,967	32.1	36	8.7
Yes	12,649	67.9	376	91.3
Atrophic gastritis and anti-H. pylori IgG status[4]				
Category A	5,396	29.0	12	2.9
Category B	5,504	29.5	104	25.3
Category C	7,145	38.4	272	66.0
Category D	571	3.1	24	5.8

[1]Median (interquartile range).
[2]Atrophic gastritis was defined as a pepsinogen I level $\leq$70 ng/mL combined with a pepsinogen I/II ratio $\leq$3.0.
[3]Anti-*H. pylori* IgG seropositivity was defined as a level of serum IgG $\geq$10 U/mL.
[4]Category A: Atrophic gastritis (AG) negative ([−]) and *H. pylori* antibodies (HPA) [−], Category B: AG [−]/HPA positive ([+]), Category C: AG [+]/HPA [+], Category D: AG [+]/HPA [−].

covariates was tested and included if appropriate. Non-proportionality of the covariates included in the final model was tested by the Grambsch and Therneau test.[30] Age was defined as the age in years at the beginning of the follow-up and was used as a continuous variable. Smoking was defined as current smokers compared with never or past smokers. Highly salted food consumption was defined as consumption of salted fish roe at least sometimes compared with never. Family history of gastric cancer was defined as the presence of at least one case of gastric cancer among the parents or siblings.

To estimate the cumulative probability and to model non-proportional effects, we then used a fully parametric model.[31,32] The logarithm of the baseline hazard was described by a parametric B-spline of time and non-proportional effects were modelled through the inclusion of interaction terms with the logarithm of the baseline hazard. The selection of the appropriate function of time for the baseline hazard was based on the AIC.

The predictive performance of the final model was assessed in terms of discrimination and calibration.[33] Discrimination was estimated using the generalization of Harrell's c-index[34,35] to non-proportional hazard models described by Antolini *et al.*[36] using 10 years as the reference follow-up time. Internal validation was provided by estimating the optimism-corrected c-index: 500 bootstrap samples were generated; for each of these, we performed the aforementioned two-step modelling procedure. Using the estimated parameters from the selected model, the c-index was calculated for the bootstrap sample and estimated for the original dataset. The optimism was then estimated as the average of the difference between the two c-index values over all the bootstrap samples. Finally, the optimism-corrected c-index was obtained by subtracting the estimated optimism

Table 2. Summary of the parameter estimates of the survival model developed in the whole study population of 19,028 individuals from the JPHC Cohort II

	Coefficient (SE)	Hazard ratios (95% CI)
Effect of age[1]		
Men		
5 years of follow-up	–	1.09 [1.06;1.11][2,3]
10 years of follow-up	–	1.07 [1.05;1.09]
15 years of follow-up	–	1.05 [1.02;1.08]
Women	–	
5 years of follow-up	–	1.05 [1.03;1.08]
10 years of follow-up	–	1.03 [1.01;1.06]
15 years of follow-up	–	1.01 [0.99;1.04]
Gender (men)[4]	0.777 (0.126)	2.18 [1.70;2.78]
Smoking status (current)	0.310 (0.120)	1.36 [1.08;1.72]
Fish roe, fish gut consumption	0.248 (0.104)	1.28 [1.04;1.57]
Family history	0.313 (0.169)	1.37 [0.98;1.90]
Atrophic gastritis and *H. pylori* infection status[5]		
Category A	–	Ref
Category B	2.025 (0.305)	7.58 [4.16;13.79]
Category C	2.629 (0.296)	13.86 [7.76;24.75]
Category D	2.646 (0.355)	14.09 [7.03;28.26]

[1]The effect of age being time-dependent and gender-specific (*i.e.*, the model includes an interaction term between age and gender), we do not present the model coefficients because they do not have a straightforward interpretation. Instead, we present a few hazard ratios obtained separately for men and women for different follow-up times.
[2]Hazard ratio for a 1-year increment of age.
[3]95% confidence intervals were obtained by sampling 100,000 times in the multivariate normal distribution of the coefficients of the parametric survival model and taking the 2.5th and 97.5th percentiles of the empirical hazard ratio distribution generated.
[4]Effect of gender for individuals aged 58 years.
[5]Category A: Atrophic gastritis (AG) negative ([−]) and *H. pylori* antibodies (HPA) [−], Category B: AG [−]/HPA positive ([+]), Category C: AG [+]/HPA [+], Category D: AG [+]/HPA [−].
Abbreviations: SE: standard error; CI: confidence interval.

from the apparent c-index (*i.e.*, that obtained with the original model on the original dataset).[34] Calibration was assessed through the analogue of Hosmer–Lemeshow's χ^2 test for survival analysis developed by d'Agostino and Nam[37] using 10 years as the endpoint and partitioning the study population into deciles of predicted risk.

Using the obtained parametric survival model, we estimated the age-, gender- and category-specific 10-year cumulative probability of occurrence of gastric cancer for the various combinations of smoking status, family history of gastric cancer and consumption of highly salted food. Corresponding 95% confidence intervals (CIs) were estimated by taking the 2.5th and 97.5th percentiles of the empirical 10-year cumulative probability distribution obtained by sampling 100,000 times from the distribution of the model parameters and calculating the corresponding cumulative probability estimates. Moreover, to provide a simple tool allowing clinicians and patients to estimate the risk of cancer without the need for actual survival probability computation, we developed a simple scoring system based on the final model using the method described by Sullivan *et al.*[38] We calculated the proportion of individuals in the highest tertile of predicted score who developed gastric cancer during the first 10 years of follow-up in order to provide a measure of sensitivity of the score. Similarly, specificity was assessed by estimating the proportion of individuals not classified in the highest tertile of predicted score who did not develop gastric cancer within 10 years.[39]

All analyses were performed with the SAS software (version 9.3; SAS Institute, Cary, NC) and the R statistical software (version 2.15.0; R Development Core Team, 2012).

Results

Among the study population of 19,028 individuals, a total of 412 cases of gastric cancer occurred during 270,854 person-years of follow-up, corresponding to an annual incidence rate of 1.52‰ (95% CI: 1.38, 1.67). The baseline characteristics of the subjects are summarized in Table 1.

Table 2 summarizes the results of the parametric survival regression procedure. The final model included age and gender, as well as their interaction, smoking status, highly salted food consumption, family history of gastric cancer and the

Table 3. Ten-year probability (expressed in %) of gastric cancer occurrence in men according to age, clinical characteristics and *H. pylori* infection and serum pepsinogen status

		Men								
Risk factors	Age	Category A[1]	Category B	Category C	Category D	Risk factors	Category A	Category B	Category C	Category D
None	40	0.04 [0.02;0.09]	0.31 [0.19;0.54]	0.57 [0.34;0.98]	0.58 [0.30;1.14]	Currently smoking	0.06 [0.03;0.13]	0.43 [0.23;0.80]	0.79 [0.43;1.46]	0.80 [0.39;1.67]
	50	0.09 [0.05;0.18]	0.70 [0.48;1.02]	1.28 [0.89;1.84]	1.30 [0.77;2.21]		0.13 [0.06;0.26]	0.96 [0.59;1.56]	1.75 [1.09;2.79]	1.77 [0.96;3.26]
	60	0.21 [0.11;0.38]	1.58 [1.20;2.07]	2.86 [2.26;3.63]	2.91 [1.87;4.53]		0.29 [0.15;0.56]	2.15 [1.43;3.24]	3.90 [2.66;5.69]	3.96 [2.31;6.75]
	70	0.48 [0.26;0.88]	3.56 [2.63;4.82]	6.41 [4.97;8.27]	6.51 [4.18;10.09]		0.65 [0.33;1.30]	4.83 [3.16;7.39]	8.66 [5.89;12.67]	8.80 [5.17;14.79]
Salted roe	40	0.05 [0.03;0.11]	0.40 [0.24;0.68]	0.74 [0.44;1.24]	0.75 [0.39;1.44]	Family history	0.06 [0.03;0.12]	0.43 [0.25;0.74]	0.78 [0.46;1.34]	0.80 [0.41;1.55]
	50	0.12 [0.06;0.23]	0.90 [0.63;1.29]	1.64 [1.17;2.30]	1.66 [0.99;2.79]		0.13 [0.07;0.24]	0.96 [0.65;1.41]	1.74 [1.21;2.52]	1.77 [1.04;3.01]
	60	0.27 [0.15;0.49]	2.02 [1.56;2.61]	3.65 [2.95;4.52]	3.72 [2.41;5.72]		0.29 [0.16;0.52]	2.14 [1.61;2.86]	3.89 [3.02;4.99]	3.95 [2.52;6.16]
	70	0.61 [0.33;1.13]	4.53 [3.37;6.11]	8.14 [6.39;10.37]	8.27 [5.35;12.71]		0.65 [0.35;1.21]	4.82 [3.50;6.63]	8.64 [6.59;11.32]	8.78 [5.62;13.60]
Currently smoking + salted roe	40	0.07 [0.03;0.16]	0.55 [0.30;1.01]	1.01 [0.56;1.82]	1.02 [0.50;2.10]	Currently smoking + family history	0.08 [0.03;0.18]	0.59 [0.32;1.10]	1.07 [0.58;1.99]	1.09 [0.52;2.26]
	50	0.16 [0.08;0.33]	1.23 [0.77;1.95]	2.23 [1.43;3.49]	2.27 [1.25;4.11]		0.17 [0.08;0.36]	1.30 [0.80;2.14]	2.37 [1.48;3.82]	2.41 [1.31;4.44]
	60	0.37 [0.19;0.72]	2.75 [1.86;4.05]	4.96 [3.48;7.06]	5.05 [2.99;8.45]		0.39 [0.20;0.77]	2.92 [1.92;4.44]	5.27 [3.57;7.76]	5.36 [3.12;9.14]
	70	0.83 [0.42;1.65]	6.15 [4.08;9.26]	10.96 [7.62;15.69]	11.13 [6.64;18.38]		0.89 [0.44;1.78]	6.53 [4.22;10.07]	11.62 [7.84;17.11]	11.80 [6.92;19.73]
Salted roe + family history	40	0.07 [0.03;0.15]	0.55 [0.33;0.92]	1.00 [0.61;1.67]	1.02 [0.54;1.94]	All	0.10 [0.04;0.22]	0.75 [0.42;1.36]	1.37 [0.77;2.46]	1.39 [0.69;2.83]
	50	0.16 [0.09;0.31]	1.22 [0.86;1.74]	2.22 [1.60;3.10]	2.26 [1.36;3.75]		0.22 [0.11;0.45]	1.67 [1.05;2.64]	3.03 [1.96;4.69]	3.08 [1.71;5.52]
	60	0.37 [0.20;0.66]	2.74 [2.12;3.53]	4.95 [4.03;6.08]	5.03 [3.29;7.66]		0.50 [0.26;0.97]	3.72 [2.53;5.47]	6.71 [4.73;9.48]	6.82 [4.07;11.29]
	70	0.83 [0.45;1.54]	6.13 [4.57;8.23]	10.93 [8.62;13.85]	11.10 [7.25;16.85]		1.14 [0.57;2.25]	8.29 [5.51;12.43]	14.64 [10.23;20.78]	14.87 [8.96;24.14]

[1]Category A: Atrophic gastritis (AG) negative ([−])/*H. pylori* infection (HPA) [−], Category B: AG [−]/HPA positive ([+]), Category C: AG [+]/HPA [+], Category D: AG [+]/HPA [−]. 95% confidence intervals were obtained by sampling 100,000 times in the multivariate normal distribution of the model parameter estimates and taking the 2.5th and 97.5th percentiles of the empirical distribution of the 10-year probability of gastric cancer occurrence estimates generated.

Table 4. Ten-year probability (expressed in %) of gastric cancer occurrence in women according to age, clinical characteristics and *H. pylori* infection and serum pepsinogen status

Women										
Risk factors	Age	Category A[1]	Category B	Category C	Category D	Risk factors	Category A	Category B	Category C	Category D
None	40	0.03 [0.02;0.07]	0.26 [0.15;0.44]	0.47 [0.28;0.80]	0.48 [0.25;0.93]	Currently smoking	0.05 [0.02;0.11]	0.35 [0.19;0.66]	0.64 [0.35;1.19]	0.65 [0.32;1.36]
	50	0.06 [0.03;0.10]	0.42 [0.29;0.60]	0.76 [0.54;1.07]	0.78 [0.46;1.30]		0.08 [0.04;0.15]	0.57 [0.36;0.92]	1.04 [0.66;1.64]	1.06 [0.58;1.93]
	60	0.09 [0.05;0.16]	0.68 [0.52;0.89]	1.24 [1.00;1.55]	1.27 [0.81;1.97]		0.12 [0.06;0.24]	0.93 [0.62;1.40]	1.70 [1.17;2.47]	1.73 [1.01;2.95]
	70	0.15 [0.08;0.28]	1.13 [0.80;1.59]	2.05 [1.52;2.76]	2.08 [1.29;3.36]		0.20 [0.10;0.41]	1.54 [0.97;2.43]	2.79 [1.83;4.25]	2.84 [1.61;4.99]
Salted roe	40	0.04 [0.02;0.09]	0.33 [0.20;0.56]	0.60 [0.37;1.01]	0.61 [0.32;1.18]	Family history	0.05 [0.02;0.10]	0.35 [0.20;0.64]	0.64 [0.36;1.15]	0.65 [0.33;1.32]
	50	0.07 [0.04;0.13]	0.53 [0.38;0.76]	0.98 [0.71;1.34]	0.99 [0.60;1.65]		0.08 [0.04;0.15]	0.57 [0.37;0.88]	1.04 [0.69;1.57]	1.06 [0.60;1.85]
	60	0.12 [0.06;0.21]	0.87 [0.68;1.13]	1.59 [1.30;1.95]	1.62 [1.05;2.50]		0.12 [0.07;0.23]	0.93 [0.65;1.33]	1.69 [1.23;2.34]	1.72 [1.05;2.82]
	70	0.19 [0.10;0.36]	1.44 [1.03;2.03]	2.62 [1.95;3.52]	2.66 [1.65;4.29]		0.20 [0.10;0.40]	1.53 [1.01;2.32]	2.78 [1.91;4.06]	2.83 [1.67;4.79]
Currently smoking + salted roe	40	0.06 [0.03;0.13)	0.45 [0.25;0.83]	0.82 [0.46;1.49]	0.84 [0.41;1.71]	Currently smoking + family history	0.06 [0.03;0.15]	0.48 [0.25;0.94]	0.88 [0.46;1.69]	0.89 [0.42;1.92]
	50	0.10 [0.05;0.20]	0.73 [0.47;1.15]	1.33 [0.87;2.05]	1.35 [0.75;2.43]		0.10 [0.05;0.22]	0.78 [0.46;1.33]	1.42 [0.85;2.36]	1.44 [0.76;2.74]
	60	0.16 [0.08;0.31]	1.19 [0.81;1.76]	2.17 [1.53;3.09]	2.21 [1.30;3.72]		0.17 [0.08;0.34]	1.27 [0.79;2.03]	2.31 [1.49;3.58]	2.35 [1.31;4.19]
	70	0.26 [0.13;0.53]	1.96 [1.26;3.08]	3.56 [2.37;5.36]	3.62 [2.07;6.31]		0.28 [0.13;0.58]	2.09 [1.25;3.49]	3.79 [2.34;6.11]	3.85 [2.09;7.05]
Salted roe + family history	40	0.06 [0.03;0.13]	0.45 [0.26;0.80]	0.82 [0.48;1.43]	0.84 [0.43;1.65]	All	0.08 [0.04;0.19]	0.62 [0.33;1.17]	1.12 [0.61;2.10]	1.14 [0.55;2.40]
	50	0.10 [0.05;0.19]	0.73 [0.49;1.10]	1.33 [0.91;1.95]	1.35 [0.78;2.32]		0.13 [0.06;0.28]	0.99 [0.60;1.65]	1.81 [1.13;2.92]	1.84 [0.99;3.41]
	60	0.16 [0.08;0.30]	1.19 [0.85;1.66]	2.17 [1.62;2.90]	2.20 [1.36;3.55]		0.22 [0.11;0.43]	1.62 [1.04;2.53]	2.95 [1.96;4.44]	3.00 [1.71;5.24]
	70	0.26 [0.13;0.51]	1.96 [1.31;2.93]	3.55 [2.49;5.09]	3.61 [2.16;6.05]		0.36 [0.17;0.74]	2.67 [1.63;4.38]	4.83 [3.06;7.61]	4.91 [2.71;8.81]

[1]Category A: Atrophic gastritis (AG) negative ([−])/*H. pylori* infection (HPA) [−], Category B: AG [−]/HPA positive ([+]), Category C: AG [+]/HPA [+]; Category D: AG [+]/HPA [−]. 95% confidence intervals were obtained by sampling 100,000 times in the multivariate normal distribution of the model parameter estimates and taking the 2.5th and 97.5th percentiles of the empirical distribution of the 10-year probability of gastric cancer occurrence estimates generated.

Table 5. Equations derived from the parametric survival regression model and used to estimate the 10-year probability of cancer occurrence according to the pattern of exposure to the included risk factors

10-year cumulative probability of gastric cancer occurrence as a function of the vector of risk factors X = *(Age, Sex, Smoking, Salt, Family, Category)*.

$$\mathrm{CumPr}(10, X) = 1 - S(10, Age)^{\exp(\mathrm{PredLin}(X))}$$

with[1]

$$S(t, Age) = \exp\left(-\frac{16.88 \cdot \exp(-9.4832)}{0.2417 - 0.0593\,(Age-58)}\left(\exp\left(\frac{0.2417 - 0.0593 \cdot (Age-58)}{16.88}t\right) - 1\right)\right)$$

and

$$\begin{aligned}\mathrm{PredLin}(X) = {} & 0.0675 \cdot (Age-58) + 0.7773 \cdot Sex + 0.0321 \cdot (Age-58) \cdot Sex + 0.3102 \\ & \cdot Smoking + 0.2478 \cdot Salt + 0.3130 \cdot Family + 2.0251 \cdot (Category=B) \\ & + 2.6289 \cdot (Category=C) + 2.6456 \cdot (Category=D)\end{aligned}$$

where:

- *Age* is the age (in years) at the beginning of the follow-up;
- *Sex* is 0 for women and 1 for men;
- *Smoking* is 0 for never and past smokers and 1 for current smokers;
- *Salt* is 0 for individuals who do not eat fish roe and 1 otherwise;
- *Family* is 0 for individuals with no family history of gastric cancer (parents or siblings) and 1 otherwise;
- (*Category* = "*i*") takes the value 1 if the individual is in category "*i*" and the value 0 otherwise.

[1]It should be noted that the survival function takes a particularly simple form in this case because the baseline hazard was modelled with a linear B-spline (hence, the survival function has an analytical form). In general, when using B-splines to model the baseline hazard, the estimation of the survival function at a particular point in time requires numerical integration.

categories defined by the anti-*H. pylori* IgG and serum pepsinogen statuses. The logarithm of the baseline hazard was modelled by a linear B-spline and the effect of age was modelled as linear and non-proportional. The two most important risk factors of gastric cancer occurrence were atrophic gastritis [category D, HR (hazard ratio) = 14.09 (7.03, 28.26)] and *H. pylori* infection [category B, HR = 7.58 (4.16, 13.79)]. Individuals positive for both atrophic gastritis and *H. pylori* infection (category C) had a risk very similar to that of individuals with atrophic gastritis only [category D, HR = 13.86 (7.76, 24.75)].

The developed prediction model showed good predictive performance. The generalized c-index at 10 years was estimated at 0.777 (optimism-corrected value: 0.768). The calibration analysis revealed reasonably good agreement between the observed and predicted number of gastric cancer cases in groups defined by the deciles of the predicted risk distribution (Nam and d'Agostino's χ^2 test: 14.78; p value = 0.06; see Supporting Information Fig. S1).

Tables 3 and 4 summarize the sex- and category-specific 10-year cumulative probability estimates for various combinations of the other risk factors. The 10-year probability of gastric cancer occurrence was generally higher in men than in women, with estimates varying from 0.04 to 14.87% for men and from 0.03 to 4.91% for women. Age had an important effect in men, with a more than fivefold increase for men aged 60 years and a more than tenfold increase at age 70 years compared with men aged 40 years (*e.g.*, 1.58 and 3.56% *vs.* 0.31%, respectively, for men in category B without any of the three other risk factors). For women, the impact of age was less important, with a 2.5-fold increase at age 60 years and a fourfold increase at age 70 years compared with women aged 40 years (*e.g.*, 1.70 and 2.79% *vs.* 0.64%, respectively, for women in category C who are current smokers). Ten-year probability estimates were also found to vary greatly with category. Estimates ranged from 0.04 to 1.14% for men (0.03 to 0.36% for women) in category A and from 0.58 to 14.87% for men (0.48 to 4.91% for women) in category D. These results can be obtained by using the equation provided in Table 5.

The simplified scoring system derived from the described risk prediction model is presented in Table 6. Based on their age, category membership and status regarding the three risk factors, individuals can be assigned a score ranging from 0 to 24. Individuals with a score of 10 or less have a predicted 10-

Table 6. Simplified scoring system for estimating the 10-year cumulative probability of gastric cancer occurrence based on the survival regression model developed

Age (Women)	
40-44	0
45-49	1
50-54	2
55-59	3
60-64	4
65-71	5

Age (Men)	
40-44	1
45-49	3
50-54	4
55-59	6
60-64	8
65-71	10

Family history	
No	0
Yes	1

Smoking status	
Never, past	0
Current	1

Salted roe	
No	0
Yes	1

Category	
A	0
B	8
C	11
D	11

Total	/24

Score	10-year probability (%)
0-10	≤0.4
11	0.6
12	0.7
13	0.9
14	1.2

Score	10-year probability (%)
15	1.5
16	1.9
17	2.5
18	3.2
19	4.1

Score	10-year probability (%)
20	5.2
21	6.6
22	8.3
23	10.6
24	13.4

year cumulative probability of gastric cancer occurrence of less than 0.4%, while this probability is >5% for individuals with a score of 20 or more. Sensitivity, as assessed by the proportion of the individuals who developed gastric cancer during the first 10 years of follow-up in the highest tertile of predicted score (score of 16 or more), was 69.7%. Specificity, as assessed by the proportion of individuals free from the disease during the first 10 years of follow-up who were not classified in the highest tertile of predicted score, was 70.0%. By comparison, using the original ABC categories without any adjustment and defining positivity as being in categories B, C or D, we estimated the sensibility to be 96.5% and the specificity, 28.8%.

Discussion

In this work, we developed a prediction model for estimating the cumulative probability of gastric cancer occurrence based on a cohort of 19,028 individuals combining demographic and clinical variables (age, gender, smoking status, consumption of highly salted food and family history of gastric cancer) as well as biological information (serum anti-*H. pylori* IgG titres, levels of pepsinogen I and pepsinogen II). The model showed good performance in terms of discrimination and calibration.

In agreement with previous studies, we found that *H. pylori* infection and atrophic gastritis were important risk factors of gastric cancer occurrence. However, in contrast to Watabe

et al.[10] we did not find any substantial difference between the risk of individuals in categories C and D, suggesting that the distinction between these two categories has little impact on the estimated risk of gastric cancer occurrence. These results are consistent with those of a recent meta-analysis of studies conducted in East Asian populations.[40]

Tatemichi *et al.*[41] suggested that the cut-off level used for defining negativity of the serum anti-*H. pylori* antibody titre was too high, so that a substantial number of individuals from category C were wrongly classified as belonging to category D. Indeed, they found that odds ratios for category D were much higher than those of category C when reducing the anti-*H. pylori* IgG titre cut-off point to 5 U/mL, suggesting the use of a more stringent level to define negativity of anti-*H. pylori* antibody titres. This could explain our failure to find a difference between hazard ratios in the C and D categories.

Another concern related to the choice of the cut-off level used for defining negativity of the serum anti-*H. pylori* antibody titre is that some individuals in category A may be in fact infected with *H. pylori* and thus may have their risk of gastric cancer occurrence underestimated. A comparison of the crude annual gastric cancer incidence rate in category A for individuals with an anti-*H. pylori* antibody titre of <5 U/mL *vs.* greater or equal to 5 U/mL revealed no significant difference in our study population (0.162 *vs.* 0.098‰, respectively, $p = 0.52$).

However, the aim of our study was to provide estimates of the risk of gastric cancer occurrence associated with the ABC categories—in conjunction with clinical and lifestyle-related factors—as defined by the criteria already existing and commonly used in routine. This, in particular, ensures the comparability of our results with those of previous studies.[10,40] It should also be emphasized that the strategy of determining an optimal cut-off point for an explanatory variable based on the outcome is known to suffer from important biases in the estimation of the effect of the variable as well as in the associated significance test.[42,43]

Our model also showed an important effect of age, particularly in men, and smoking and highly salted food consumption were found to be independent predictors of gastric cancer occurrence. Even if the impact of these latter two risk factors was found to be very limited for individuals in category A, they modified substantially the cumulative probability of gastric cancer occurrence in other categories, particularly in older men. While the inclusion of these lifestyle-related risk factors does not justify a modification of the recommendations for gastric cancer surveillance based on the ABC method, the quantification of the actual cumulative probabilities of gastric cancer occurrence associated with exposure to these factors might provide an incentive for adopting healthier lifestyles, particularly for high-risk individuals. Nonetheless, we also provided in the Supporting Information the results obtained with a minimal model including age, gender and the ABC categories (see Supporting Information Tables S1 and S2) that can be used when information regarding lifestyle habits and family history is not available.

Our study has several strengths. First, the population used in this work is the largest cohort with available information for both *H. pylori* infection status and serologically defined atrophic gastritis, as well as information on lifestyle characteristics. This allowed us to confirm the results of previous studies and to provide reliable estimates of the risk factor effects. Second, the use of a flexible parametric model[31,32] enabled us to give a complete description of the survival model used to estimate the cumulative probability for a given follow-up time. This is particularly interesting because it allows other researchers to conduct an external validation including the assessment of the model calibration.[44]

The major limitation of this work is the absence of external validation of our prediction model. Although the model showed good properties in terms of discrimination and calibration and limited optimism because of the large sample size, the generalizability of our results cannot be ascertained without testing the model on an independent dataset.[44,45] Several mechanisms might have an influence on the generalizability of our results. In particular, there might be differences in risk estimates between the study population and the target (general) population arising from differences in the distribution of unmeasured or unknown confounders. However, this concern might be partly alleviated by the fact that our model included all risk factors known to have a strong relationship with gastric cancer occurrence in the Japanese population. It is also possible that, even in the absence of residual confounding, the estimated effects of the risk factors differ significantly from those of the target population.[46] This problem might be limited here by the fact that we used a large sample and that the relationship between risk factors and gastric occurrence is based on probable underlying biological mechanisms thought to be similar in the study and the general population. Be that as it may, external validation presupposes the existence of an appropriate risk prediction model. Because this work represents the first large-scale attempt to combine biological, clinical and lifestyle-related risk factors for predicting the risk of gastric cancer in the Japanese population, we focused here on the development of an appropriate model. Further work is thus needed to validate the results of our study and confidently extend its use to the general Japanese population.

Another limitation pertains to the fact that we defined highly salted food consumption as the occasional consumption of salted fish roe, which is unlikely to account for the real consumption of highly salted food items. In a study using a different questionnaire in a different population, we showed that salted fish roe and salted fish preserves were strongly associated with gastric cancer.[14] However, we remarked that these findings could be explained by a preference for highly salted food in individuals eating those food items in general rather than a specific effect of those items on the risk of gastric cancer occurrence. Moreover, in the preliminary assessment of nutritional variables for our study, we found a

consistent effect of salted roe consumption that was not dose-dependent, which also suggests that this variable may act as a marker of inclination for highly salted food items.

Finally, we did not consider subtypes of gastric cancer as defined by the classical subdivision between cardia and non-cardia gastric cancers. These cancers are associated with different risk factors, with cardia gastric cancer sharing risk factors with oesophageal adenocarcinomas.[5] Until recently, no distinction was made in Japan between gastric cancers localized in the cardia or in the upper third of the stomach, making it impossible to distinguish cardia from noncardia cancers. However, cases coded C160 (cardia) or C161 (fungus) represented only 8.3% of all gastric cancer cases that occurred in the study population, so the impact of not having considered subtypes in our analyses might have been limited.

In conclusion, we developed a risk prediction model for gastric cancer based on clinical and lifestyle-related characteristics in combination with biological variables that allows the estimation of the 10-year probability of gastric cancer occurrence. This model and the simple scoring system derived from it might provide incentive for individuals to modify their lifestyle habits, attend regular check-up visits or participate in screening programmes.

APPENDIX

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References

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer, 2013. Available at: http://globocan.iarc.fr (Accessed September 3, 2014).
2. Inoue M, Tsugane S. Epidemiology of gastric cancer in Japan. *Postgrad Med J* 2005;81:419–24.
3. Cancer Statistics in Japan, 2014. Available at: http://ganjoho.jp/public/statistics/pub/statistics01.html (Accessed September 18, 2014).
4. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345:784–9.
5. Conteduca V, Sansonno D, Lauletta G, et al. *H. pylori* infection and gastric cancer: state of the art (review). *Int J Oncol* 2013;42:5–18.

6. de Martel C, Ferlay J, Franceschi S, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol* 2012;13:607–15.
7. Plummer M, Franceschi S, Vignat J, et al. Global burden of gastric cancer attributable to *Helicobacter pylori*. *Int J Cancer* 2015;136:487–90.
8. Brenner H, Arndt V, Stegmaier C, et al. Is *Helicobacter pylori* infection a necessary condition for noncardia gastric cancer? *Am J Epidemiol* 2004; 159:252–8.
9. Miki K. Gastric cancer screening by combined assay for serum anti-*Helicobacter pylori* IgG antibody and serum pepsinogen levels–"ABC method." *Proc Jpn Acad Ser B Phys Biol Sci* 2011;87: 405–14.
10. Watabe H, Mitsushima T, Yamaji Y, et al. Predicting the development of gastric cancer from combining *Helicobacter pylori* antibodies and serum pepsinogen status: a prospective endoscopic cohort study. *Gut* 2005;54:764.
11. Brenner H, Arndt V, Bode G, et al. Risk of gastric cancer among smokers infected with *Helicobacter pylori*. *Int J Cancer* 2002;98:446–9.
12. Sasazuki S, Sasaki S, Tsugane S; Japan Public Health Center Study Group. Cigarette smoking, alcohol consumption and subsequent gastric cancer risk by subsite and histologic type. *Int J Cancer* 2002;101:560.
13. Nishino Y, Inoue M, Tsuji I, et al.; Research Group for the Development Evaluation of Cancer Prevention Strategies in Japan. Tobacco smoking and gastric cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol* 2006;36:800–7.
14. Tsugane S, Sasazuki S, Kobayashi M, et al. Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women. *Br J Cancer* 2004;90:128–34.
15. Kobayashi M, Tsubono Y, Sasazuki S, et al.; JPHC Study Group. Vegetables, fruit and risk of gastric cancer in Japan: a 10-year follow-up of the JPHC Study Cohort I. *Int J Cancer* 2002;102: 39–44.
16. Shimazu T, Wakai K, Tamakoshi A, et al.; Research Group for the Development Evaluation of Cancer Prevention Strategies in Japan. Association of vegetable and fruit intake with gastric cancer risk among Japanese: a pooled analysis of four cohort studies. *Ann Oncol* 2014;25:1228–33.
17. Tsugane S, Sasazuki S. Diet and the risk of gastric cancer: review of epidemiological evidence. *Gastric Cancer* 2007;10:75–83.
18. Buckland G, Travier N, Huerta JM, et al. Healthy lifestyle index and risk of gastric adenocarcinoma in the EPIC cohort study. *Int J Cancer* 2015;137: 598–606.
19. Lee TY, Wang CB, Chen TT, et al.; Taiwan Gastrointestinal Disease Helicobacter Consortium. A tool to predict risk for gastric cancer in patients with peptic ulcer disease on the basis of a nationwide cohort. *Clin Gastroenterol Hepatol* 2015;13: 287–93.
20. Tata MD, Gurunathan R, Palayan K. MARK's quadrant scoring system: a symptom-based targeted screening tool for gastric cancer. *Ann Gastroenterol* 2014;27:34–41.
21. Mizuno S, Miki I, Ishida T, et al. Prescreening of a high-risk group for gastric cancer by serologically determined *Helicobacter pylori* infection and atrophic gastritis. *Dig Dis Sci* 2010;55:3132–7.
22. Yoshida T, Kato J, Inoue I, et al. Cancer development based on chronic active gastritis and resulting gastric atrophy as assessed by serum levels of pepsinogen and *Helicobacter pylori* antibody titer. *Int J Cancer* 2014;134:1445–57.
23. Hamashima C, Shibuya D, Yamazaki H, et al. The Japanese guidelines for gastric cancer screening. *Jpn J Clin Oncol* 2008;38:259–67.
24. Suenaga I, Sasazuki S, Tsugane S. Further study of translational research for preventive medicine. *Prev Med* 2012;55:573–4.
25. Tsugane S, Sobue T. Baseline survey of JPHC study-design and participation rate. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. *J Epidemiol* 2001;11: S24–S29.
26. World Health Organization. International classification of diseases for oncology, 3rd edn. Geneva, Switzerland: World Health Organization, 2000. 240 p.
27. Tramacere I, Negri E, Pelucchi C, et al. A meta-analysis on alcohol drinking and gastric cancer risk. *Ann Oncol* 2012;23:28–36.
28. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition, physical activity and the prevention of cancer: a global perspective. Washington, DC: AICR, 2007. 517 p.
29. Yaghoobi M, Bijarchi R, Narod SA. Family history and the risk of gastric cancer. *Br J Cancer* 2010;102:237–42.
30. Grambsch PM, Therneau TM. Proportional hazard tests and diagnostics based on weighted residuals. *Biometrika* 1994;81:515–26.
31. Remontet L, Bossard N, Belot A, et al.; French Network of Cancer Registries FRANCIM. An overall strategy based on regression models to estimate relative survival and model the effects of prognostic factors in cancer survival studies. *Stat Med* 2007;26:2214–28.
32. Charvat H, Sasazuki S, Inoue M, et al.; JPHC Study Group. Impact of five modifiable lifestyle habits on the probability of cancer occurrence in a Japanese population-based cohort: results from the JPHC study. *Prev Med* 2013;57:685–9.
33. Moons KG, Kengne AP, Woodward M, et al. Risk prediction models: I. Development, internal validation, and assessing the incremental value of a new (bio)marker. *Heart* 2012;98:683–90.
34. Harrell FE, Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996;15:361–87.
35. Pencina MJ, d'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. *Stat Med* 2004;23:2109–23.
36. Antolini L, Boracchi P, Biganzoli E. A time-dependent discrimination index for survival data. *Stat Med* 2005;24:3927–44.
37. D'Agostino RB, Nam BH. Evaluation of the performance of survival analysis models: discrimination and calibration measures. In: Balakrishnan N, Rao CR, eds. Handbook of statistics, vol. 23. Amsterdam: Elsevier, 2004. 1–25.
38. Sullivan LM, Massaro JM, d'Agostino RB. Sr. Presentation of multivariate data for clinical use: the Framingham Study risk score functions. *Stat Med* 2004;23:1631–60.
39. D'Agostino RB, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care. *Circulation* 2008;117:743–53.
40. Terasawa T, Nishida H, Kato K, et al. Prediction of gastric cancer development by serum pepsinogen test and *Helicobacter pylori* seropositivity in Eastern Asians: a systematic review and meta-analysis. *PLoS One* 2014;9:e109783.
41. Tatemichi M, Sasazuki S, Inoue M, et al.; JPHC Study Group. Clinical significance of IgG antibody titer against *Helicobacter pylori*. *Helicobacter* 2009;14:231–6.
42. Altman DG, Lausen B, Sauerbrei W, et al. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. *J Natl Cancer Inst* 1994;86:829–35.
43. Lausen B, Schumacher M. Evaluating the effect of optimized cutoff values in the assessment of prognostic factors. *Comput Stat Data Anal* 1996; 21:307–26.
44. Royston P, Altman DG. External validation of a Cox prognostic model: principles and methods. *BMC Med Res Methodol* 2013;13:33.
45. Moons KG, Kengne AP, Grobbee DE, et al. Risk prediction models: II. External validation, model updating, and impact assessment. *Heart* 2012;98: 691–8.
46. Iwasaki M, Yamamoto S, Otani T, et al.; Japan Public Health Center-based Prospective Study Group. Generalizability of relative risk estimates from a well-defined population to a general population. *Eur J Epidemiol* 2006;21:253–62.

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LETTER TO THE EDITOR

Reactive oxygen species and gastric cancer risk: a large nested case–control study in Japan

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Reactive oxygen species (ROS) is a term used to describe a number of reactive molecules and free radicals derived from molecular oxygen [1]. ROS are produced in normal biological processes in cells by means of either enzymatic or nonenzymatic mechanisms and play beneficial and adverse roles in an organism [2]. Oxidative stress, an imbalance toward the pro-oxidative state, is caused by the presence of free radicals or radical-generating agents in concentrations that overwhelm natural radical-blocking or radical-scavenging mechanisms [1]. Under oxidative stress conditions, excessive ROS can damage DNA, cellular proteins, and lipids, leading to fatal lesions in cells that contribute to carcinogenesis [3].

The ROS include superoxide, hydrogen peroxide, hydroxyl radical, hydroxyl ion, and nitric oxide [1]. *Helicobacter pylori* infection was regarded as a human gastric carcinogen by the International Agency for Research on Cancer, and the ROS level was higher in *H. pylori*–positive than in *H. pylori*–negative patients with gastritis [4]. However, *H. pylori* infection is not a sufficient factor for development of gastric cancer. Previous studies have reported that serum ROS levels among smokers increased with the number of cigarettes smoked per day [2]. Other environmental factors such as alcohol intake, obesity, and chemicals also increase the ROS level [3, 5–7].

We conducted a case–control study nested within the Japan Public Health Center–based Prospective Cohort (JPHC) Study to investigate total serum levels of ROS in relation to subsequent development of gastric cancer and focused on exogenous factors of lifestyle.

The JPHC Study, included 140,420 subjects defined as inhabitants of the study areas who were 40 years of age or older, was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan. In 1990 (for cohort I) and in 1993–1994 (for cohort II), subjects were asked to reply to a lifestyle questionnaire including sociodemographic factors (Fig. 1). Among eligible participants, 36,745 subjects (38 %) each voluntarily donated 10-mL blood samples at health check-ups conducted by each PHC [8]. Subjects were asked to avoid eating a meal later than 2100 hours (9:00 pm) the day before the examination. The last time of either eating a meal or drinking water or tea was documented. The plasma and buffy layer were divided into four tubes, each holding 1.0 mL (three tubes for plasma and one for the buffy layer), and then stored at −80 °C.

The JPHC study was followed-up until 31 December 2004. Newly diagnosed cases of cancer were collected from active patient notifications from the local major hospitals in the study area and data linkage with population-based registries (prefecture-wide), on the basis of site by International Classification of Diseases for Oncology (ICD-O) code C160-169. During the entire study period, 512 new gastric cancer cases were identified.

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Histologic classification was based on the review of the record reported by each hospital [8]. For each case, one control was selected from subjects who had no history of gastric cancer and who lived in the study area when the case was diagnosed. Each control was matched to a case for gender, age (±3 years), study area, blood donation date (±2 months), and fasting time at blood donation (±5 h). Because of a technical error in measurement of *H. pylori* (one pair) and inadequate volume of blood available for ROS measurements (six pairs), seven pairs of subjects were excluded. Finally, we had 495 sets each of cases and controls for use in the present analysis.

The ROS assay was established by modifying the assay system for derivatives of reactive oxygen metabolites (D-ROM), which had a high correlation coefficient of 0.94 with those measured by the conventional D-ROM test, and the coefficients of variation were 2.4 to 2.5 % for within-run reproducibility and 3.2 to 4.3 % for between-day reproducibility [2]. Immunoglobulin (Ig) G antibodies to *H. pylori* were measured with a direct enzyme-linked immunosorbent assay kit (E Plate "Eiken" *H. pylori* antibody; Eiken Kagaku Co. Ltd, Tokyo, Japan). Levels of IgG were categorized as seropositive or seronegative for *H. pylori* according to a selective cut-off value (492 nm). Assays of cytotoxin-associated gene A (CagA) were performed using an enzyme-linked immunosorbent assay kit in which horseradish peroxidase was used as the enzyme tracer (CagA IgG EIA; Sceti Co. Ltd, Rome, Italy). Serum levels of pepsinogen I (PGI) and pepsinogen II (PGII) were measured by commercial kits based on a two-step enzyme immunoassay (E Plate "Eiken" Pepsinogen I; Eiken Kagaku Co. Ltd; and E Plate "Eiken" Pepsinogen II; Eiken Kagaku Co. Ltd). Results were defined as atrophy when the criteria of both PGI level ≤70 ng/mL and PGI: PGII ratio ≤3.0 were fulfilled [8]. All measurements were conducted by a person blinded to the case–control situation.

Conditional logistic regression was used to measure the association between ROS level and gastric cancer risk for all paired study subjects, with multiple adjustments for potential confounding factors, including smoking status, alcohol consumption, intake of salt, body mass index (BMI), family history of gastric cancer, history of diabetes, *H. pylori* infection, and atrophy. Family history of gastric cancer was regarded as positive if at least one parent or

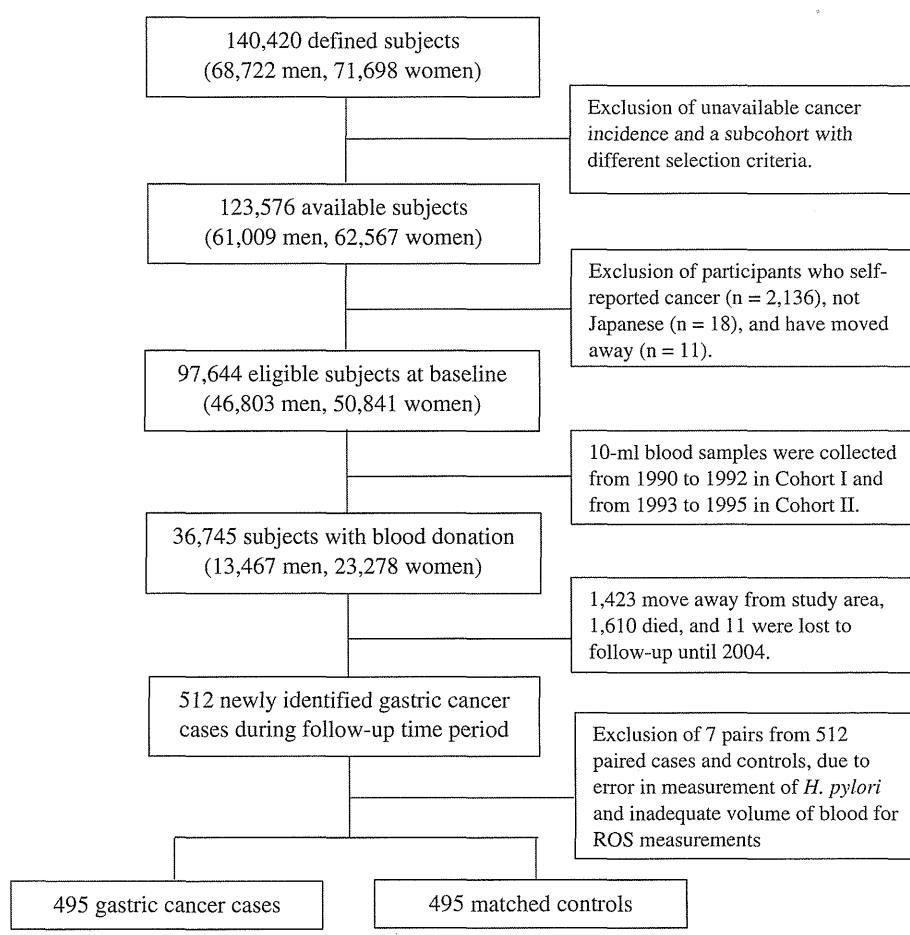

Fig. 1 Flowchart on selected study subjects and identified gastric cancer cases controls during follow-up time between 1990 and 2004. ROS, reactive oxidant species

sibling had gastric cancer. ROS level was categorized based on the distribution of control subjects. Odds ratios (ORs) were calculated relative to the lowest quintile of serum ROS level. Unconditional logistic regression was applied for subjects stratified by smoking status (never/ever), alcohol consumption (no/yes), BMI (<25/≥25 kg/m^2), *H. pylori* infection (negative/positive), CagA seropositivity (negative/positive), or atrophy status (no/yes).

The mean (SD) of study subjects were 57.4 (7.2) years of age. Compared with controls, cases had more predominant positivity of *H. pylori* (93.9 vs. 75.0 %, $P < 0.0001$), CagA (75.6 vs. 70.3 %, $P = 0.063$), atrophy (81.8 vs. 58.4 %, $P < 0.0001$), family history of gastric cancer (11.7 vs. 8.1 %, $P = 0.016$), and history of diabetes (9.3 vs. 4.7 %, $P < 0.01$). There were more overweight (BMI ≥25 kg/m^2) subjects in the control group (25.3 %) than in the case group (19.8 %, $P = 0.04$).

The medians of total ROS levels were 120 units (interquartile range 36) in the case group and 119 units (interquartile range 34) in the control groups ($P = 0.94$). There were no significant associations with risk of development of gastric cancer in subjects in the highest quintile compared with the lowest quintile of ROS levels (Table 1). The same analysis was performed for subjects without pairs with cases diagnosed during the first 2 (14 pairs) or 3 years (61 pairs) of follow-up and for paired subjects in each tertile of follow-up years (<4.33/4.33 to <7.95/≥7.95) based on all subjects; no significant associations were observed.

Multivariate analysis results with adjustment for lifestyle and infection factors showed that significant associations between ROS levels and gastric cancer risk were seen in the highest quintile in ever-smokers and alcohol drinkers. Significantly reduced risks of development of gastric cancer were observed in the highest quintile of ROS levels in women, never-smokers, and nondrinkers of alcohol in the model with adjustment for lifestyle factors, but not in the model with adjustment for both lifestyle and infection factors. There were no significant joint effects of total ROS and sex, smoking status, alcohol consumption, or BMI associated with gastric cancer development.

To our knowledge, this is the first prospective, cohort-nested, case–control study to have investigated the association between total ROS level and the risk of gastric cancer. Excessive ROS production in gastric epithelial cells might be triggered and might enhance gastric carcinogenesis. Our study supports the notion that exogenous ROS-modulating agents are likely to cause elevations in ROS above the toxic threshold level, leading to cell death [5].

There are several measurements to reflect ROS production. The improved assay system based on the principle of D-ROM allows high-throughput and automated analysis of numerous serum samples in terms of high reproducibility, consistent validity, and much smaller amounts of sera and regents than the conventional D-ROM test [2].

In the *H. pylori*–infected gastric mucosa, high ROS (i.e., hypochlorous acid [HOCL] and hydroxyl radicals [·OH]) cannot eradicate bacteria and the phagocytes produce more ROS [5]. Also, *H. pylori* has various defense mechanisms against external ROS attacks to protect against elimination [5]. Although the bacterium is categorized as a definite carcinogen, it is not always associated with increased risk of gastric cancer development. Infection with pathogenic bacteria other than *H. pylori* can induce cancer-promoting events, resulting in further damage to adenocarcinoma cells in the stomach [6]. In our study, the similar levels in *H. pylori*–positive and *H. pylori*–negative groups of subjects may indicate that other sources of ROS (endogenous and exogenous) were also critical in damaging gastric mucosa when total ROS reached a certain level.

In a systematic review of a Japanese population, the increased gastric cancer risk for tobacco users is convincing evidence [9], whereas evidence of increased risk of gastric cancer attributable to alcohol consumption is insufficient [10]. Ethanol metabolism is directly involved in and facilitates ROS production and cytokine release and forms an in vivo oxidative microenvironment, thus creating suitable conditions for the development of pathologies directly related to oxidative stress [3]. After ethanol intoxication, the balance between pro-oxidants and antioxidants is disturbed, thus resulting in oxidative damage of biomolecules, including fats, proteins, and DNA, and finally leading to cell injury [3]. Our study results show that the higher incidence of risk of gastric cancer was found in alcohol drinkers, and the higher ROS levels may support this notion and help clarify the evidence of the risk of gastric cancer attributable to alcohol intake.

Our study showed increased risk in those with BMI <25 kg/m^2 and decreased risk in those with BMI ≥25 kg/m^2 regarding gastric cancer associated with ROS levels, although this was not statistically significant. It had been reported that mild oxidative stress caused by moderate physical activity can activate cellular stress response signaling and potential cellular antioxidant defense ability [7]. Physical activity regulates a greater flux of oxygen free radicals that could alter cellular redox status, depending on the type, intensity, duration, and frequency of physical activity and the type of diet consumed [7]. Because we did not have comprehensive information regarding physical