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JOHNS
特集●検査結果をどう読むか？●

細菌・ウイルス検査

HIV 関連の検査

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Keiko YODA

● Key Words ● HIV，スクリーニング検査，確定検査，AIDS ●

Ⅰ．HIV 検査の意義

抗 HIV 薬の開発と，これを用いた抗 HIV 療法 ART（antiretroviral therapy）の導入によって，HIV 感染症の治療は大きく進歩している。AIDS による死亡数は減少し，HIV 感染者は長期生存が可能となった。世界全体の新規 HIV 感染者は 1998 年をピークに減少傾向にあるが[1]，わが国における新規 HIV 感染者数は 2008 年の 1,126 件をピークに 2007 年以降は毎年 1,000 件/年以上[2]の高止まりの状態で，先進国のなかで最も対策が遅れている国と言わざるを得ない。

ART は HIV を排除する根治的な方法ではないが，早期の ART が HIV 感染者の予後をより改善することを示した知見が示され，年々 ART の開始が早まっている[3]。早期の診断・治療は，HIV 感染者の予後の改善のみならず新たな感染を防ぐことにもつながるため，HIV 感染の早期診断がこれまで以上に重要視されている。

Ⅱ．HIV 感染症の自然経過

HIV 感染症は，ウイルスが免疫担当細胞（主と

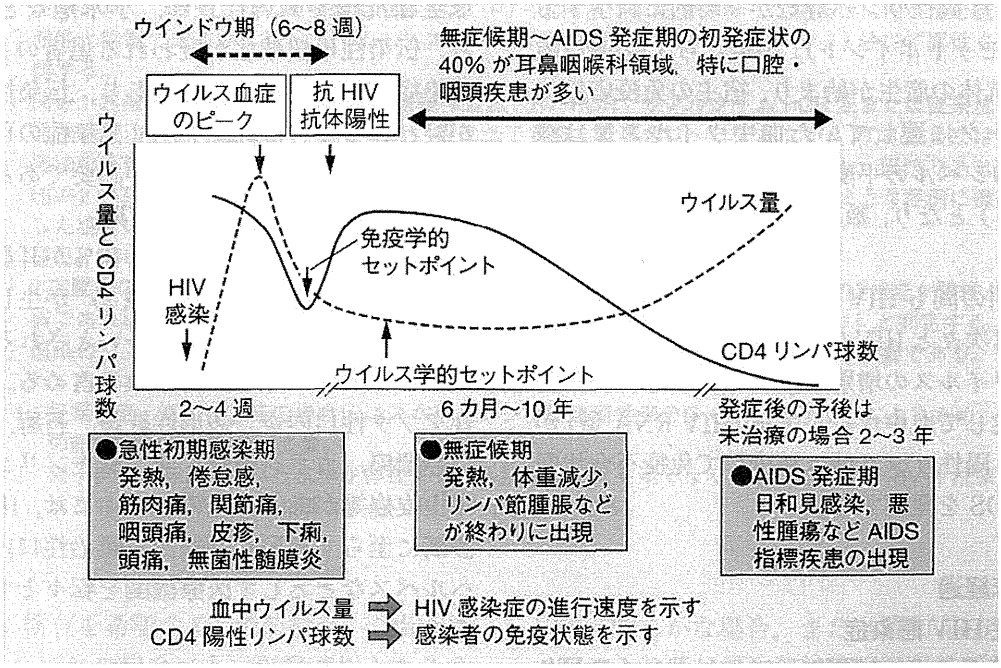

図 1　HIV-1 感染症の自然経過（IDWR 感染症発生動向調査週報，感染症の話）
2002 年第 40 週号（2002 年 9 月 30 日〜10 月 6 日）掲載，後天性免疫不全症候群（後編）
（http://idsc.nih.go.jp/idwr/kansen/k02_g2/k02_40/k02_40.html より引用，一部改正）

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表 1　急性 HIV-感染症の症状（文献 3 より引用）

・急性 HIV 感染症を疑う：最近（2〜6 週間以内）あった HIV 曝露危険度の高い行動に引き続く兆候あるいは症状
以下の兆候・症状臨床検査所見が単独あるいは複合してみられる 発熱（96%），リンパ節腫脹（74%），咽頭炎（70%），皮疹（70%），筋肉痛・関節痛（54%），頭痛（32%），下痢（32%），嘔気・嘔吐（27%）など*
HIV 曝露危険度の高い行動とは，HIV 感染者あるいは HIV 感染のリスクを有する人との性的接触，麻薬静注などにおける注射器などの共有，HIV が含まれる可能性がある体液への粘膜などの曝露があげられる
・鑑別診断：EBV および非 EBV（CMV など）感染による伝染性単核球症，インフルエンザ，ウイルス性肝炎，連鎖球菌感染症，梅毒など

＊：Ann Intern Med 137：381, 2002

して CD4 陽性リンパ球）に感染し，免疫系が徐々に破壊されながら進行する疾患である。未治療の HIV 感染者の経過は，感染初期（急性期），無症候期，AIDS 発症期の順に進行する（図 1）。

1．免疫学的経過

HIV 感染成立直後から血中で HIV は急速に増殖し，4〜6 週後に血中ウイルス量がピークに達する。この時期が感染初期（急性期）で，感染者の約 50〜90％に何らかの症状が出現する。並行して HIV が感染した CD4 陽性リンパ球も破壊されるために，CD4 陽性リンパ球数が一時的に減少する（免疫学的セットポイント）。感染から 6〜8 週目頃より HIV 抗体の産生が始まり，宿主の免疫応答によってピークに達していた血中ウイルス量は減少，一定のレベルの平衡状態（ウイルス学的セットポイント）となり，数年から 10 年ほどの無症候期に入る。

無症候期の間も HIV の増殖は続き，CD4 陽性リンパ球は次々と HIV に感染して死滅していく。やがて，ウイルスの増殖と宿主の免疫応答の平衡状態が破綻して血中ウイルス量（HIV RNA 量）が増加，CD4 陽性リンパ球は減少して免疫不全状態となり AIDS を発症する。

2．臨床経過

1）急性 HIV 感染症

HIV 感染から 2〜6 週後，血中ウイルス量が急増する感染初期の約 50〜90％の感染者にみられる。発熱，倦怠感，筋肉痛，リンパ節腫脹，発疹（**表 1**）といったインフルエンザ様の症状が主で[3]，

表 2　HIV 感染に関連する口腔病変（文献 5 より引用）

感染症	真菌感染，細菌感染，ウイルス感染
新生物	カポジ肉腫，非ホジキンリンパ腫，扁平上皮癌
炎症性	再発性アフタ性口内炎，多形性紅斑，苔癬
原因不明	唾液腺疾患，非特異的口腔潰瘍，メラニン色素の過度の沈着

程度は軽症から無菌性髄膜炎に至る重症までさまざまで，2〜3 週間以内に自然に消退する。発疹は体幹，顔面，手掌，足底などに麻疹や伝染性単核球症様皮疹や鱗屑性丘疹，小水疱などがみられる。伝染性単核球症が疑われる患者の 2％は HIV 感染症であるという報告もあり，伝染性単核球症が疑われる場合は，急性 HIV 感染症の可能性を考慮し，注意深い観察と検査が必要である[4]。

2）無症候期・AIDS 発症期

無症候期以降，初発症状の 40％が耳鼻咽喉科領域，特に口腔咽頭に病変（**表 2**）[5]が生じ，それらが診断の契機となる場合が多いとされる。最も多いのがカンジダ症で，約半数を占める。他，再発性アフタ性口内炎，多形性紅斑，苔癬，非特異的口腔潰瘍，カポジ肉腫，非ホジキンリンパ腫，扁平上皮癌等がある。感染者の中には，HIV 感染の診断に至らず風邪や咽頭炎・難治性口内炎・口腔ヘルペスなどとして医療機関を転々とする場合がある。

Ⅲ．HIV 感染の診断

1．対象者

HIV 検査は，医療者側から HIV 感染を疑う，患

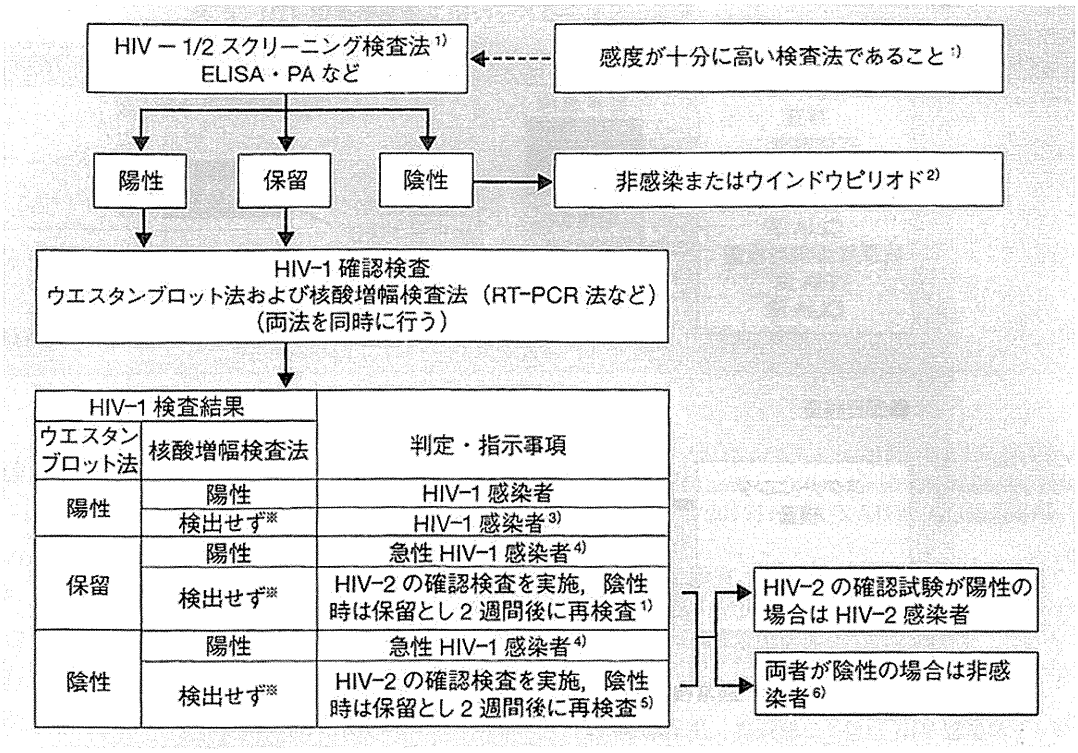

図 2　診療における HIV-1/2 感染症診断のためのフローチャート（文献 8 より引用）

1）明らかな感染のリスクがある場合や急性感染を疑う症状がある場合は抗原・抗体同時検査法によるスクリーニング検査に加え HIV-1 核酸増幅検査法による検査も考慮する必要がある（ただし，現時点では保険適用がない）。

2）急性感染を疑って検査し，HIV-1/2 スクリーニング検査とウエスタンブロット法が陰性または保留であり，しかも，HIV-1 核酸増幅検査法（RT-PCR 法）が陽性であった場合は，HIV-1 の急性感染と診断できるが，後日，HIV-1/2 スクリーニング検査とウエスタンブロット法にて陽性を確認する。

3）HIV-1 感染者とするが，HIV-1 核酸増幅検査法（RT-PCR：リアルタイム PCR 法または従来法の通常感度法）で「検出せず※」の場合（従来法で実施した場合は，リアルタイム PCR 法または従来法の高感度法における再確認を推奨）は HIV-2 ウエスタンブロット法を実施し，陽性であれば HIV-2 の感染者であることが否定できない（交差反応が認められるため）。このような症例に遭遇した場合は，専門医，専門機関に相談することを推奨する。

4）後日，適切な時期にウエスタンブロット法で陽性を確認する。

5）2 週間後の再検査において，スクリーニング検査が陰性であるか，HIV-1/2 の確認検査が陰性/保留であれば，初回のスクリーニング検査は偽陽性であり，「非感染（感染はない）」と判定する。

6）感染のリスクがある場合や急性感染を疑う症状がある場合は保留として再検査が必要である。また，同様な症状をきたす他の原因も平行して検索する必要がある。

注 1：妊婦健診，術前検査等の場合にはスクリーニング検査陽性例の多くが偽陽性反応によるため，その結果説明には注意が必要。

注 2：母子感染の診断は，移行抗体が存在するため抗体検査は有用でなく，児の血液中の HIV-1 抗原，または HIV-1 核酸増幅検査法により確認する必要がある。

者自ら HIV 検査を希望する，術前検査，の場面に行われる。いずれの場合でも，保険適用となるのは，① AIDS 指標疾患との鑑別が難しい疾病が認められる場合，②HIV 感染に関連しやすい性感染症が認められる場合，③非加熱凝固因子製剤の投与歴が明らかな場合，または昭和 53 年から 63 年の間に入院歴があり非加熱凝固因子製剤投与の可能性が否定できない場合[6]である。術前検査として HIV 検査を行う場合は保険適用とならないが，感染対策として費用を医療者側が負担して実施し

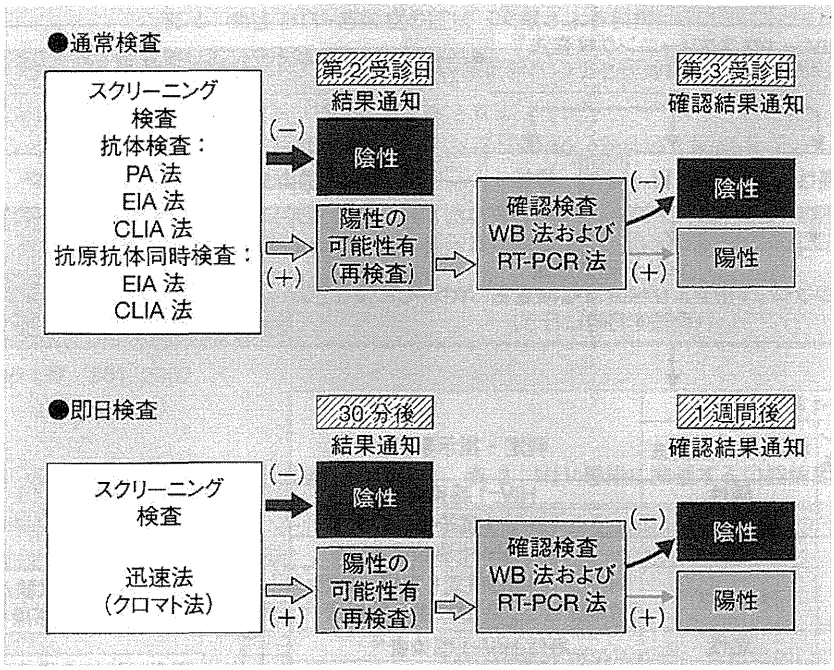

図 3　通常検査と即日検査の流れ（文献 3 より引用）

ている施設は少なくない。また，希望者は各都道府県の保健所等で，無料で HIV 検査が受けられる[7]。

AIDS 発症前の HIV 感染症には，特異的症状や所見はない。HIV 検査を行うことではじめて診断される。HIV 感染の早期発見のため，すべての医療者は受診者の症状やリスクに注意を払い，HIV の早期診断に努めることが求められている[3,6]。わが国における新規 HIV 感染者の 90％が日本国籍男性で，約 70％は 20 歳～40 歳台の男性同性愛者である[2]。男性同性愛者は，淋菌，クラミジア，肝炎などの性感染症に感染するリスクの高い性行動を持つ場合が多く，特に梅毒の陽性率が高い。他に，帯状疱疹，痔，口腔咽頭カンジダ症を認めるか，または既往がある，臨床所見やその治療経過が臨床経験上“普段みている症例と何か違う”印象を受ける患者が，20歳～40歳台の男性の場合は HIV 感染症である可能性を考慮して対応するべきである。

検査を行う際，医学的に必要性があるにも関わらず意識障害などのために本人の同意が得られない場合を除き，すべての場合において前もって本人に説明し同意を得なければならない[6]。

2．診断に用いられる検査

通常，①スクリーニング検査，②確認検査，の順で行われ，確認検査が陽性の場合に診断が確定する。診断には，日本エイズ学会と日本臨床検査医学会が作成したガイドラインに従って行う（**図 2**）[8]。

1）スクリーニング検査

通常検査として PA 法（粒子凝集法），ELISA 法（酵素抗体法），CLIA 法（化学発光免疫測定法）がある。感染初期（急性期）の可能性が否定できない場合は，ELISA 法または CLIA 法で抗原抗体同時検査を行う。スクリーニング検査では，感染者を見落とさない高感度の方法を採用することが重要で，比較的簡単で精度の高い ELISA 法が普及している。一方，一部の保健所や医療機関では，即日検査として 15 分で結果が得られるイムノクロマトグラフ法が行われている。スクリーニング検査には，通常検査で 0.3％，即日検査で 1％の疑陽性が認められるため，陽性の場合は確認検査を行う（**図 3**）。HIV 陽性の結果は，本人のみならず家族やパートナーへの影響が大きいため，スクリーニング検査では疑陽性が認められることの説明も大切である。

表 3　HIV 感染症診断に用いられる各検査の特徴（文献 6 より引用）

検査の種類		検査の対象	ウインドウピリオド	その他
スクリーニング	第 1 世代	HIV-1 IgG	約 50 日	偽陽性あり
	第 2 世代	HIV-1/2 IgG		
	第 3 世代	HIV-1/2 IgG/M	最短 22 日[B]	
	第 4 世代	第 3 世代＋HIV-1 p24 抗原	最短 17 日[B]	
ウエスタンブロット法		HIV 粒子の各構成成分に対する IgG 抗体	全バンドが陽転化するまでの時間には個人差が大きい	特異度が高い
核酸増幅検査（PCR 法）		HIV-1 RNA （HIV-2 RNA については研究室レベルの検査）	約 2 週間	稀に偽陽性

2）確認検査

スクリーニング検査が陽性の場合，WB 法（western blot 法）と HIV-1 核酸増幅検査法を同時に行い，診断を確定する。

3）ウインドウ期

いずれの検査でも，HIV 感染成立後から血中の抗体量やウイルス量が検査測定閾値に達するまでの期間，検査結果が陰性となる期間が存在する（表 3）。この期間をウインドウ期（ウインドウピリオド）という。このウインドウ期には血中にフリーの HIV ウイルス粒子が多量に存在し，感染性の高い危険な時期である。スクリーニング検査が陰性でも，臨床所見と病歴からウインドウ期であることが疑われる場合は，保険適用外であるが，HIV-1 核酸増幅検査法（RT-PCR 法など）による確認を考慮する。

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High-risk human papillomavirus correlates with recurrence after laser ablation for treatment of patients with cervical intraepithelial neoplasia 3: A long-term follow-up retrospective study

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Abstract

Aim: The purpose of our study was to evaluate the efficacy of laser ablation as a conservative treatment for cervical intraepithelial neoplasia 3 (CIN3) and assess whether the human papillomavirus (HPV) test is useful to predict recurrence after treatment.

Materials and Methods: A total of 134 patients who received laser ablation for treatment of CIN3 were enrolled in this study. During the follow-up period, patients were followed with cytological and colposcopic evaluations. Recurrence of CIN3 was regarded as the primary end-point. HPV genotype was tested before and after treatment. Post-treatment cumulative recurrence rates were estimated and comparisons by both patient age and HPV genotype were performed.

Results: Overall cumulative recurrence rate of CIN3 in the first year after treatment was 22.6% for all patients. No significant correlation was shown between patient age and recurrence. Patients infected by specific genotypes (16, 18, 31, 33, 52, and 58) frequently failed to clear the infection after treatment. The 1-year recurrence-free survival in those positive after treatment for eight high-risk genotypes (16, 18, 31, 33, 35, 45, 52, and 58) was significantly lower (66.7%), compared to that in those positive for other high-risk types (78.6%). The recurrence-free survival of those who remained HPV-positive after treatment was significantly lower than those who turned negative.

Conclusion: Laser ablation should be performed prudently with appropriate patient counseling about recurrence rate. Considering its minimal invasiveness, laser ablation is effective, especially for young patients who are negative for eight high-risk genotypes. With regard to HPV testing, although genotyping has significant value for predicting recurrence, screening for all genotypes warrants further evaluation.

Key words: cervical intraepithelial neoplasia 3, human papillomavirus testing, laser ablation, recurrence, treatment efficacy.

Introduction

The spread of systematic screening programs has detected more cervical intraepithelial neoplasia (CIN) and has succeeded in producing marked declines in cervical carcinoma incidence and mortality in the developed countries where screening programs and treatment for pre-invasive lesions are

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widespread. Many women who receive treatment for high-grade CIN are of reproductive age with a mean age of approximately 30 years old.[1] Therefore, the treatment should not only be effective but also have minimum adverse effects on future fertility and obstetrical outcomes. Cold-knife conization, laser conization, loop electrosurgical excisional procedure (LEEP), cryotherapy, and laser ablation are all conservative methods used to treat high-grade CIN by removing or destroying the transformation zone containing abnormal epithelial cells and thereby preserving cervical function. According to data from the Japan Society of Obstetrics and Gynecology (JSOG), conservative conization methods were chosen for only 33% of women with carcinoma *in situ* (CIS) in 1990, for which hysterectomy had been the treatment standard, rising as high as 79.3% in 2009. These data robustly represent the increasing demand for conservative CIN treatments at the present time.

Many studies have demonstrated that these methods show similarly low morbidity and are equally successful at preventing invasive cervical cancer.[2–5] Of these conservative methods, characteristics of laser ablation have been well reported due to its fertility-sparing advantage. Laser ablation is usually performed under local anesthesia as an outpatient procedure whereas conization procedures need general anesthesia and inpatient care. Regarding pregnancy outcomes, excisional treatment procedures, including cold-knife conization, laser conization, and LEEP, are associated with increased risk of adverse obstetric morbidity. In contrast, ablative procedures, including cryotherapy and laser ablation, are free of any of these untoward outcomes.[5,6] However, resected specimens from excisional procedures allow for precise histological diagnosis, including presence of unexpected microinvasive diseases, while ablative methods, by destroying cervical tissue, preclude this investigation and require additional pre-treatment biopsy. This ability to combine diagnosis with treatment in a single procedure remains an advantage of excisional treatments.

Women with high-grade CIN frequently undergo excisional treatments because, while more invasive, they are more definitive than ablative therapies. As such, there are few reports available regarding the efficacy of ablative treatments, such as laser ablation for high-grade CIN. Moreover, most studies comparing efficacy between treatments show both the rate of recurrence and residual disease, which is the failure rate; since these study populations often include a variety of CIN1 to CIN3 patients, and definitions for recurrence/resi dual disease depend on treatments, failure rates also vary markedly between studies. Both randomized and non-randomized studies demonstrate a failure rate of 5–30% for laser ablation and 5–16% for LEEP in a 6-month follow-up period;[7,8] however, in 2002, Dey *et al.* demonstrated that the cumulative risk of cytological abnormality reported as moderate dysplasia or worse is higher after laser ablation than LEEP.[9] A recent long-term follow-up study found cryotherapy was associated with the highest rate of recurrence compared with conization, LEEP, and laser ablation.[10] In total, the treatment efficacy against CIN has been still inconsistent comparing laser ablation against other excisional methods, such as conization and LEEP, and there is little information for the safety and efficacy of laser ablation for high-grade lesion.

In this study, we propose that laser ablation is a useful modality for the treatment of CIN in terms of obstetrics outcomes, even for high-grade lesions, if satisfactory colposcopy and consecutive cytology after treatment are available. In addition, we aimed to perform a descriptive investigation of the recurrence of high-grade CIN after laser ablation. Furthermore, the International Agency for Research on Cancer (IARC) announced in 2003 that among the over 100 HPV genotypes, 13 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) should be considered carcinogenic, thus defined as 'high-risk types'.[11] Since then, HPV testing has been proposed as part of the follow-up of patients treated for high-grade CIN due to its very high sensitivity and negative predictive value for detecting residual/recurrent disease,[12] suggesting that it may be a good indicator of disease clearance. Indeed, a growing body of evidence has demonstrated that HPV testing together with cytology is useful in monitoring women treated for high-grade CIN.[12,13] To further clarify this in the setting of ablative therapy, we focused on the correlation between high-grade CIN recurrence rates and HPV genotype before and after laser ablation.

Methods

Patients

Following Japanese standard treatment protocols, in our study, those patients whose cervical biopsy demonstrated CIN3 (excluding CIS) and whose histology, cytology, and colposcopy were in concordance, were

treated with laser ablation between 2004 and 2010 at the Tokyo University Hospital. Patients were followed up with cytology and colposcopy 3 months after laser ablation, and those who showed residual disease were excluded from this study. Patients with negative cytology and normal colposcopic findings 3 months after treatment were included in this retrospective study. A total of 144 patients (mean age, 36.9 years; range, 25–71 years) met these criteria and were included in this retrospective study. Patients were followed up for at least 5 years with cytological and colposcopic evaluations conducted at intervals of 3–4 months. No residual lesion was confirmed by satisfactory colposcopic findings with negative cytology on the ecto-endocervix together. The recurrence of CIN3 was regarded as the primary end-point. Referring to the previous publications,[9,10] date of recurrence was defined as the mid-point between the date of the examination when abnormal cytology or histology (such as moderate-severe dysplasia, atypical squamous cells that cannot exclude HSIL [ASC-H], or high-grade squamous intraepithelial lesion [HSIL]) was first detected with satisfactory colposcopy, and the most recent preceding examination in which the colposcopic evaluations and smear (ecto-endocervix) were normal.

In total, 83 patients (median age, 36 years) were further examined for the efficacy of laser ablation by HPV genotypes, identified by polymerase chain reaction (PCR)-based HPV DNA testing before and after ablation, comparing the post-treatment persistent infection and recurrence-free survival (RFS) rates. HPV genotyping was performed in each patient. Regarding the natural history of CIN in Japan, a recent prospective cohort study by Matsumoto *et al.*[14] showed that the cumulative progression rate for CIN3 within 5 years was 20.5% for eight types of high-risk HPV (16, 18, 31, 33, 35, 45, 52, and 58), which was significantly higher than the 6.0% observed for five other high-risk types (39, 51, 56, 59, and 68), demonstrating that differences in progression exists even in the 13 HPV types defined by IARC as high-risk. In our study, therefore, we classified the study population according to this report and focused on the eight 'higher-risk' types. Informed consent was obtained in all cases. The median follow-up period was 17 months, with a minimum of at least 6 months. Recurrence was defined as emergence of CIN3 in complete responders.

PCR-based HPV DNA testing

DNA was extracted from cervical smear samples by using the QIAGEN DNeasy Blood & Tissue Kits. PCR-based HPV DNA testing was performed using the PGMY-CHUV assay. Briefly, standard PCR was conducted using the PGMY09/11 L1 consensus primer sets and HLA-dQ primer sets. Reverse blotting hybridization was subsequently performed as described previously.[15]

Laser ablation

Outpatient carbon dioxide laser procedures were carried out under colposcopic guidance, taking about 10 min, without anesthesia or premedication. Water in the tissue absorbs the laser energy, which destroys tissue by vaporization. To be effective, the lesion is typically ablated to a depth of 5 mm on the ectocervix and 8–9 mm around the endocervix. After ablation, the epithelium regenerates in 2–3 weeks. All cases were performed by gynecologic oncologists using CO2 laser, MEDILASER-30S (Model mel-30S, Mochida) with a power density of 8–12W in continuous mode.

Statistics

Date of recurrence was defined as the mid-point between the date of the examination when abnormal cytology was first detected and the most recent preceding examination in which the smear was normal. The log–rank test was used to assess differences in cumulative risk between study groups; tests of significance were carried out at the 5% two-sided level.

Results

We initially identified 144 patients with CIN3 who received laser ablation at Tokyo University Hospital between 2004 and 2010, and showed both negative cytology and normal colposcopic findings 3 months after treatment. Ten patients were excluded because of incomplete data. A total of 134 cases of CIN3 (median age, 37 years; range, 27–71 years, excluding CIS) were monitored every 3–4 months during the follow-up period (6–95 months; median, 38 months). Seven (5.2%) were censored at 1 year, 19 (14.2%) at 2 years, and 105 (78.4%) at 5 years after treatment. The recurrence of CIN3 was regarded as the primary end-point. All the patients were evaluated with satisfactory colposcopy and histological examination of transitional zone.

First, we investigated the efficacy of laser ablation against all CIN cases. During the follow-up period, recurrence was identified in 57 (42.5%) of the 134 patients, and the overall cumulative CIN3 recurrence rate in the first 12 months after treatment was 22.6%

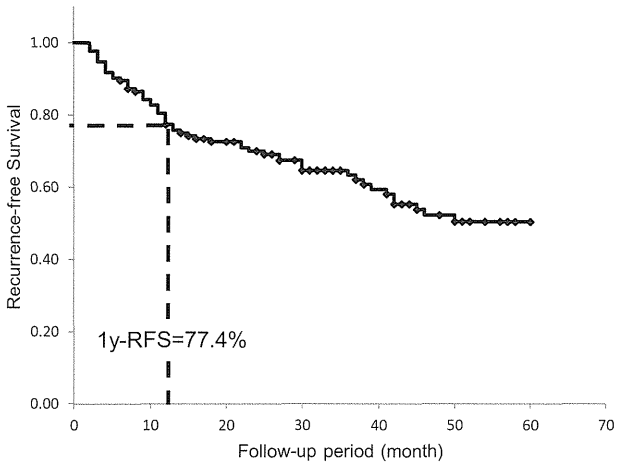

Figure 1 Overall cumulative recurrence rate after treatment. The overall cumulative recurrence rate of cervical intraepithelial neoplasia 3 in the first 12 months after treatment was 22.6%. RFS, recurrence-free survival.

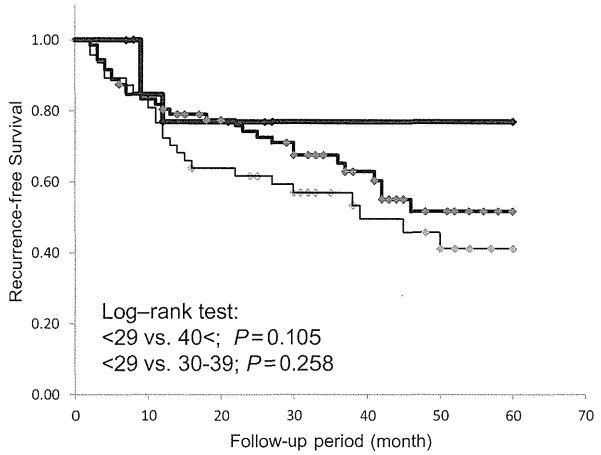

Figure 2 Recurrence rates compared by age. No significant elevated risk of recurrence was seen between younger age (<29) and older age (<29 vs >40; $P = 0.105$, <29 vs 30–39; $P = 0.258$, by log–rank test). ▬, <29; ▬, 30–39; ▬, 40<.

(Fig. 1). Figure 2 shows recurrence rates compared by age. It is worth noting that no significant elevated risk of recurrence was seen between younger age (<29) and older age (<29 vs >40, $P = 0.105$; <29 vs 30–39, $P = 0.258$, by log–rank test). Among recurrence cases, 27 patients underwent second laser ablation, nine patients underwent cervical conization, and one patient underwent total hysterectomy. Other patients were followed up with intensive cytological and colposcopic evaluations

Table 1 Distribution of HPV type before treatment

HPV type before treatment	Rate (%)
16	34
18	2
31	2
33	5
52	20
58	13
Others	24

The HPV genotypes of 83 patients were identified by polymerase chain reaction-based HPV DNA testing both before and after ablation. Seventy-six women were positive on HPV-DNA testing (91.6%). The distribution of HPV genotypes was classified according to the Matsumoto criteria of 8 'higher-risk' types. Two of the higher-risk types (35, 45) were not detected in this study. All cervical intraepithelial neoplasia 3 cases in this study had monoinfection. HPV, human papillomavirus.

with informed consent. The re-recurrence rate of CIN3 after second ablation was 11.1%.

Next, we evaluated the association between HPV genotype and efficacy of laser ablation, assessing whether HPV genotyping might predict failure after laser ablation. Of the 134 patients, we examined the HPV genotype of 83 patients, identified by PCR-based HPV DNA testing both before and after ablation. Single infection was observed in all CIN3 cases in this study, although there is a possibility that some patients might have multiple infections, especially in the CIN1-2 population. The median follow-up period was 17 months, with a minimum follow-up of 6 months. The median age of these 83 patients was 36 years; 76 women were positive on HPV-DNA testing (91.6%). The distribution of HPV genotypes, classified according to the Matsumoto criteria of eight 'higher-risk' types, before laser ablation is shown in Table 1.

Table 2 shows detection rates – that is, whether the same HPV type was detected before and after laser ablation, which can be interpreted as a good indicator of disease clearance. HPV was persistently detected after treatment more often in the higher-risk types, especially in type 16 and 18, than in other HPV types (16/18 vs 31/33/52/58 vs others; 66.6% vs 52.4% vs 25.0%; $P < 0.0001$ by Cochran–Armitage test).

In addition, we compared post-treatment RFS by HPV genotypes. As shown in Figure 3, we first compared RFS by pre-treatment HPV genotypes, that is, between those who were positive for the 8 higher-risk types and those positive for other HPV types before ablation; no statistically significant difference was seen between the two ($P = 0.77$ by log–rank test). We then

Table 2 Detection rate of HPV after treatment for each HPV type before treatment

HPV type before treatment	Detection of HPV after treatment†	
	Positive (%)	Negative (%)
16/18	66.6	33.3
31/33/52/58	52.4	47.6
Others	25.0	75.0

†Cochran–Armitage test $P < 0.0001$. Detection rates – i.e. whether the same HPV type was detected before and after laser ablation, which can be interpreted as a good indicator of disease clearance. HPV was persistently detected after treatment more often in the higher-risk types, especially in types 16 and 18, than in other HPV types (16/18 vs 31/33/52/58 vs others; 66.6% vs 52.4% vs 25.0%; $P < 0.0001$ by Cochran–Armitage test). HPV, human papillomavirus.

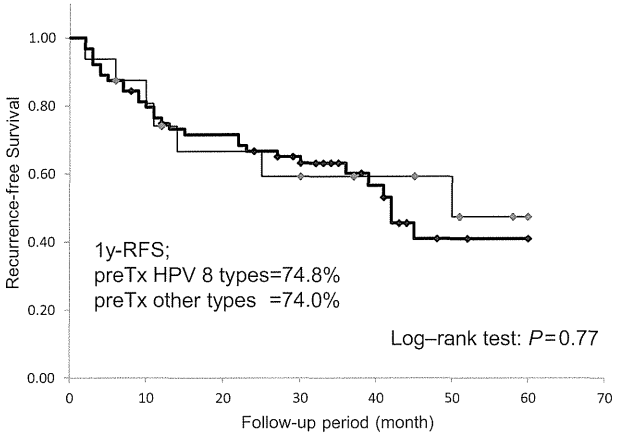

Figure 3 Recurrence-free survival (RFS) by pre-treatment human papillomavirus (HPV) genotype. We compared RFS between those who were positive for the 8 higher-risk types versus those positive for other HPV types prior to ablation; no significant difference between the two groups was identified. —, 8 types; —, Others.

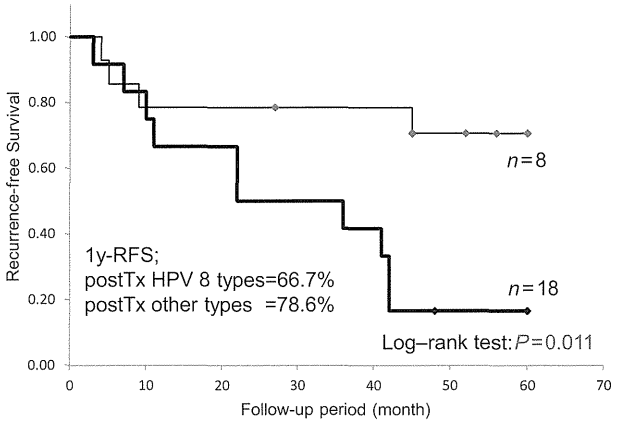

Figure 4 Recurrence-free survival (RFS) by post-treatment human papillomavirus (HPV) genotype. After ablation, 1-year RFS was 66.7% for those who were positive for the 8 higher-risk HPV types, which was significantly lower than the 78.6% observed in those positive for other HPV types. —, 8 types; —, Others.

compared RFS by post-treatment HPV genotypes. As shown in Figure 4, 1-year RFS was 66.7% for those who were positive for the 8 higher-risk types after ablation, which was significantly lower than the 78.6% observed in those positive for other HPV types ($P = 0.011$ by log–rank test). Furthermore, Figure 5 shows that the 1-year RFS for those positive for all high-risk 13 types of HPV after treatment was 61.1%, versus 100% in HPV-negative subjects ($P = 0.0013$ by log–rank test).

All these data suggest that the persistence of high-risk HPV genotypes, especially of the eight types, might play a key role in the development of recurrence.

Discussion

In 2011, a large Dutch multi-cohort study, one of the representative prospective studies on long-term efficacy of CIN treatment, reported on recurrence risk after treatment for CIN2/3.[16] According to that study, the recurrence risk of CIN was quite low after highly successful treatment followed by normal cytology or negative HPV testing. However, all participants were treated by excisional methods, such as cold-knife conization or LEEP, so the data did not present any information on the prognostic outcomes of conservative treatments, including laser ablation.

As mentioned, many studies have demonstrated that conservative methods, including cold-knife conization, LEEP, cryotherapy, and laser ablation, show similarly low morbidity and are equally successful at preventing invasive cervical cancer.[4,17,18] However, in 2009, Melnikow *et al.*[10] compared the long-term follow-up of 37 142 patients with CIN by treatment modality (cold-knife conization, LEEP, laser [conization and ablation

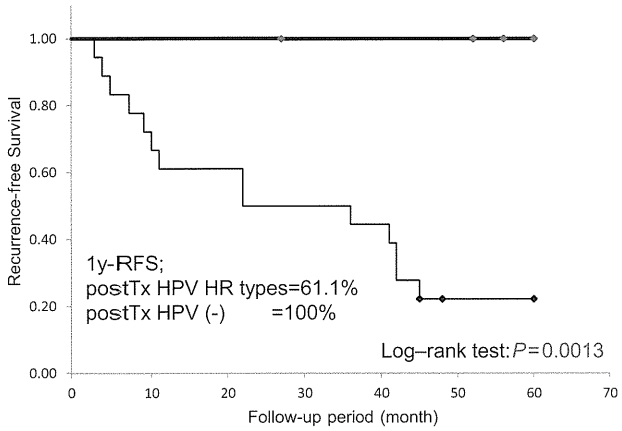

Figure 5 Recurrence-free survival (RFS) between post-treatment human papillomavirus (HPV) genotypes. Post-treatment 1-year RFS for those positive for 13 high-risk HPV was 61.1%, versus 100% for those who were HPV-negative. It is unsurprising that no recurrence was seen in any HPV-negative cases after laser ablation. —, negative; —, high-risk types.

combined], and cryotherapy), reporting that overall cumulative rate of CIN2/3 recurrence in the first 6 years after treatment of 14.0% for women originally treated for CIN3. Estimated rates of CIN2/3 after treatment were lowest for cold-knife conization, followed by LEEP and laser, and highest for cryotherapy. This result corroborates the invasiveness of the respective procedure.

Further evidences are warranted for the treatments of CIN3 though; considering many women with high-grade CIN are of reproductive age, treatment should not only be effective but also have minimum adverse effects on future fertility and obstetric outcomes. Several previous studies have clearly demonstrated that excisional treatments result in long-term adverse obstetric outcomes, including preterm delivery, low birthweight, or premature rupture of membranes; thus, an increasing number of patients are likely to choose less invasive treatment like laser ablation.[5]

The data in our study suggest that laser ablation is an optional conservative treatment for CIN3 with minimal invasiveness. Regarding obstetric outcomes, it is difficultt o follow patients after CIN treatment in terms of pregnancy because of the premalignant nature of the lesion, so no data were available in this study. Nevertheless, as laser ablation has thus far been shown to be free of any adverse effect on obstetric outcomes, it should be taken into consideration especially for young patients of child-bearing age. Even for lesions, provisional use of laser ablation may provide a 'grace period' for young women with desire to bear children before more invasive interventions are performed. Therefore, many women can postpone, or even entirely avoid, the unnecessary adverse outcomes associated with invasive intervention.

With regard to follow-up surveillance after treatment of high-grade CIN, the role of HPV-DNA testing has been an area of interest due to its high sensitivity and negative predictive value (NPV) for detecting recurrent disease. In a meta-analysis of 11 studies by Zielinski *et al.*,[12] sensitivity, NPV, and specificity of high-risk HPV testing for recurrence were 91% (95% CI = 86–95), 98% (97–99), and 79% (76–82), respectively. That study demonstrated that combining cytology with high-risk HPV testing increases rates even further: sensitivity was 96% (89–99), NPV was 99% (98–100), and specificity was 81% (77–84). Specificity is indeed relatively low, but considering that this is not a screening test but rather a follow-up for a potentially lethal disease, NPV and sensitivity should be valued higher than specificity.

We demonstrated in this study that HPV infection is not likely to disappear, but persists after laser ablation in women positive for eight 'higher-risk' HPV. Considering that cervical cancer is caused by persistent infection with a subset of carcinogenic HPV, this failure of viral clearance should lead to the increase in cervical cancer in future. Indeed, we confirmed the significant difference in RFS between those who were positive for the eight higher-risk types of post-treatment versus those who were positive for other types or altogether negative. This result supports the efficacy of HPV testing and is consistent with several previous studies.

However, several critical problems with HPV-PCR testing remain. First, while PCR testing can distinguish individual genotype, detection sensitivities differ greatly among HPV genotypes. Furthermore, routine PCR-based HPV DNA testing is not suitable for daily clinical use due to its high cost and complex technique using specific primers. For the purposes of this study context, therefore, we recommend commercially available HPV testing using the hybrid capture method, Hybrid Capture II (HC2), which targets a group of 13 high-risk HPV genotypes but does not distinguish which genotypes are present. This intensive test also represents well the eight higher-risk genotypes; identification of the existence of high-risk HPV by HC2 may sufficiently inform the likelihood of recurrence. In Japan, HC2 has been covered by insurance since 2010, but the target of this test is only limited for patients

with atypical squamous cells of undetermined significance (ASC-US) detected by screening cytology test; we recommend that HC2 should also be used widely for the surveillance of patients after treatment for CIN.

In summary, laser ablation is a useful conservative treatment for CIN3, especially for young women of reproductive age. It will continue to be necessary for gynecologists to tailor treatment strategies for young patients according to their health needs, balancing minimal adverse obstetric outcomes with minimal attenuation of efficacy. In addition, it is well established that the rate of developing high-grade disease, including invasive cervical cancer, remains elevated in these post-treatment patients, even after a very long time subsequent to treatment. Indeed, a study in 2006 warns that the rate of invasive disease remained at 56 per 100 000 woman-years, 2.8 times greater than expected, for at least 20 years after treatment.[19] Because of this substantial long-term risk, close monitoring remains critical, and combined cytological evaluation together with HPV testing should increase the safety of the surveillance. For more rigorous and detailed follow-up algorithms, including surveillance intervals, obstetric outcomes, and cost-effectiveness, larger and longer-term studies are warranted in the future.

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Disclosure

The authors have no conflicts of interest related to this article.

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Association between carotenoids and outcome of cervical intraepithelial neoplasia: a prospective cohort study

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ORIGINAL ARTICLE

Association between carotenoids and outcome of cervical intraepithelial neoplasia: a prospective cohort study

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Abstract
Background It has been suggested that micronutrients such as alpha-tocopherol, retinol, lutein, cryptoxanthin, lycopene, and alpha- and beta-carotene may help in the prevention of cervical cancer. Our aim was to investigate whether serum concentrations and/or dietary intake of micronutrients influence the regression or progression of low-grade cervical abnormalities.
Methods In a prospective cohort study of 391 patients with cervical intraepithelial neoplasia (CIN) grade 1–2 lesions, we measured serum micronutrient concentrations in addition to a self-administered questionnaire about dietary intake. We evaluated the hazard ratio (HR) adjusted for CIN grade, human papillomavirus genotype, total energy intake and smoking status.

The Japan HPV and Cervical Cancer (JHACC) Study Group.

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Results In non-smoking regression subjects, regression was significantly associated with serum levels of zeaxanthin/lutein (HR 1.25, 0.78–2.01, $p = 0.024$). This benefit was abolished in current smokers. Regression was inhibited by high serum levels of alpha-tocopherol in smokers ($p = 0.042$). In progression subjects, a significant protective effect against progression to CIN3 was observed in individuals with a medium level of serum beta-carotene [HR 0.28, 95 % confidence interval (CI) 0.11–0.71, $p = 0.007$), although any protective effect from a higher level of serum beta-carotene was weaker or abolished (HR 0.52, 95 % CI 0.24–1.13, $p = 0.098$). Increasing beta-carotene intake did not show a protective effect (HR 2.30, 95 % CI 0.97–5.42, $p = 0.058$).

Conclusions Measurements of serum levels of carotenoids suggest that regression is modulated by smoking status. Maintaining a medium serum level of beta-carotene has a protective effect for progression; however, carotene intake is not correlated with serum levels of carotenoids.

Keywords Human papillomavirus · Cervical intraepithelial neoplasia · Low-grade squamous intraepithelial lesion · Micronutrients · Carotenoids

Introduction

Persistent infection with human papillomavirus (HPV) may potentially lead to the development of cervical cancer. Most women are exposed to at least one type of genital HPV in their lifetime [1]. HPV infections often cause cervical intraepithelial neoplasia 1 (CIN1) [2]. Only a subset of individuals with CIN1 progress to CIN3 or invasive cervical cancer, suggesting that environmental cofactors are related to cervical carcinogenesis [3–5]. Numerous environmental candidates such as oral contraceptives, parity, smoking status, micronutrient status, nutrient intake, *Chlamydia trachomatis* infection and herpes simplex virus type 2 infection have been investigated as potential cofactors related to progression of CIN.

Much attention has been given to the role of dietary factors and serum micronutrients in the etiology of cervical cancer and CIN. Carotenoids and tocopherols are lipid-soluble micronutrients with potent antioxidant activities and modulatory effects on immunity. Recent publications have reported that the association of carotenoids and tocopherols with reduced risk has not been observed consistently [6–10]; however, these inconsistent results may be due to the study designs. Furthermore, the majority of case–control studies of the associations between micronutrients and outcome of CIN were conducted to assess either dietary intake or circulating micronutrients only [7–9, 11].

Foods are composites of several biologically active dietary components. Micronutrients in foods, as well as other possible anti-carcinogenic compounds such as detoxification enzymes, may have synergistic effects and interact with one another [11–13]. A recent multi-center cohort study reported an association between dietary intake of micronutrients and outcome of CIN. However, this study reported no information about circulating micronutrients [6]. Conversely, some prospective cohort studies reported an association between circulating micronutrient levels and outcome of CIN but no information about dietary intake [14, 15]. Both dietary intake and circulating serum concentrations of micronutrients are important in assessing the role of micronutrients in cervical carcinogenesis. We previously conducted a case–control study including 156 pairs of women with CIN1–3 and matched controls with normal cytology and found an inverse relationship between serum levels of alpha-carotene, lycopene and zeaxanthin/lutein and the risk of CIN development [16]. Because retrospective analysis of previous study findings provides only limited information, we report here the results of a prospective study that was conducted in an attempt to confirm these findings.

Materials and methods

Study design

We used follow-up data from the Japan HPV and Cervical Cancer Study, a prospective non-intervention cohort study conducted to identify determinants of low-grade squamous intraepithelial lesion (LSIL)/CIN regression and progression. Among a total of 570 study subjects with low-grade cervical abnormalities (cytological LSIL and histological CIN1/2) recruited from nine hospitals between 1998 and 2004, 391 women with data concerning serum micronutrients and complete entry questionnaires were enrolled in the present study. Details of the design, methods and primary results have been provided elsewhere [17, 18]. Participants entered the study only after voluntarily giving signed, informed consent. The subjects were routinely followed at 3- to 4-month intervals and received cytology and colposcopy examinations at each visit. To avoid interference of the biopsy procedure on the natural course of the disease, cervical biopsy was performed only when women had HSIL smears and major colposcopic changes that were suggestive of progression to CIN3 or worse. Progression was defined as histological CIN3 lesions or worse, diagnosed on central pathology review. We defined regression as at least two consecutive negative smears and normal colposcopy. Women were regarded as having persistent lesions when they did not have either regression or

progression over the period of follow-up. At enrollment, study subjects were tested for cervical HPV-DNA and circulating serum micronutrients. Information about smoking and dietary intake was obtained from a self-administered questionnaire. Participants were not obliged to answer the questionnaire and their participation was unrelated to their clinical evaluation, treatment or follow-up evaluation. The simplified diet history questionnaire used in the current study had been developed and validated previously [19]. Originally, a prototype diet history questionnaire including 169 traditional Japanese foods and dishes was developed. To alleviate the participants' burden, our simplified diet history questionnaire was developed to employ a stepwise regression method to select from the 169 diet history questionnaire items. This simplified questionnaire was composed of 14 categories: (1) dishes of meat and vegetables; (2) meat (without dishes including vegetables); (3) fish; (4) cereals; (5) eggs and soybean products; (6) vegetables; (7) seaweed; (8) juice; (9) fruits; (10) milk and dairy products; (11) desserts and snacks; (12) pickles; (13) seasoning; and (14) alcoholic beverages. Supplement use was not assessed in this study because of a lack of complete information regarding availability. Because it was impossible to distinguish between intake of alpha- and beta-carotene from the questionnaire, total carotene intake was described. Questions on smoking habits included status (never, former or current smoker) and intensity (number of cigarettes smoked per day).

Circulating micronutrients

Blood was collected in foil-wrapped glass tubes without heparin. Serum was separated by centrifugation at $1{,}000 \times g$ for 10 min and stored in the dark at -70 °C prior to sample preparation. Serum levels of retinol, alpha-tocopherol and various carotenoids were determined by a high-pressure liquid chromatography method described previously [21].

Statistical analysis

The association between smoking status and nutrient intake was analyzed by one-way analysis of variance. The association between smoking status and serum micronutrients was analyzed by analysis of covariance. The data were adjusted for age, body mass index (BMI) and alcohol intake frequency. For regression or progression, time to event was measured from the date of the index visit to the date of the visit at which cytological transition to normal or CIN3 was first detected. To estimate the association between the CIN outcomes and circulating serum micronutrients, serum micronutrient tertiles were examined. Hazard ratios (HRs) and 95 % confidence intervals (CIs) for each tertile with reference to the lowest tertile were calculated using a proportional hazard model. For nutrient intake, identical estimation was conducted. The Brinkman Index (BI) was calculated by multiplying the average number of cigarettes smoked per day by the smoking years. We detected HPV-DNA in exfoliated cervical cells by a PCR-based methodology described previously [20]. HPV DNA was amplified by PCR using consensus-primers (L1C1/L1C2 + L1C2M) for the HPV L1 region. HPV genotypes were identified by a restriction fragment-length polymorphism (RFLP) PCR method that has been shown to identify at least 26 genotypes of genital HPV [18]. HRs were adjusted for potential confounders, including CIN grade, HPV genotype, age, total energy intake and smoking status. Statistical analyses were performed using Stata statistical software, release 11.1 (Stata Corporation; College Station, TX, USA).

Results

Of the 570 women enrolled in the parent study, 391 met the eligibility requirements of the current study for tests of serum micronutrients and completion of entry questionnaires. Of these, 329 and 62 women were diagnosed as CIN1 and CIN2, respectively. The mean age of the women was 36.3 years (median 36.0, range 19–54). Of the 391 women, regression, persistence and progression occurred in 218, 135 and 38, respectively.

Influence of smoking status on circulating levels and intake of micronutrients

At enrollment, 190 women had never smoked, while 142 women were current smokers (BI >100). Data from three women were lost and the remaining 56 women were past smokers. We found a 22 and 10 % decrease in carotene and vitamin E intake in current smokers compared with non-smokers, respectively (Table 1). Among the three groups, there was a significant difference in the intake of fiber, calcium, carotenes, vitamin A, vitamin C and vitamin E. As shown in Table 2, current smokers had significantly lower serum levels of alpha-carotene, beta-carotene and cryptoxanthin compared with non-smokers. Smokers had marginally lower levels of lycopene. Retinol, zeaxanthin/lutein and alpha-tocopherol were not related to smoking status.

The effects of serum micronutrients and nutrient intake in regression subjects

Significantly more inhibition of regression was observed in women in the middle tertiles of serum alpha-tocopherol

Table 1 Relationship between estimated daily nutrient intake and tobacco smoking status

Nutrient intake per day	Non smokers ($N = 190$)		Past smokers ($N = 56$)		Current smokers ($N = 142$)		p value
	Mean	SD	Mean	SD	Mean	SD	
Total energy intake (kcal)	2,220.1	576.1	2,221.6	679.7	2,149.1	574.9	0.520
Protein intake (g)	85.2	26.2	85.2	31.0	79.4	27.3	0.127
Fat intake (g)	60.2	21.9	62.9	27.2	59.0	22.6	0.566
Carbohydrate intake (g)	329.5	78.3	325.2	85.6	315.2	74.6	0.255
Fiber intake (g)	5.3	1.9	5.2	2.0	4.6	1.8	0.004
Calcium intake (mg)	740.8	292.2	738.3	337.6	620.9	274.2	0.001
Retinol intake (μg)	284.6	219.1	302.4	176.9	331.2	624.7	0.597
Carotene intake (μg)	4,943.5	2,439.7	4,856.3	2,532.1	3,866.8	2,083.5	0.000
Vitamin A intake (IU)	3,430.6	1,587.5	3,424.3	1,546.9	2,954.2	2,197.4	0.049
Vitamin C intake (mg)	134.0	65.6	133.3	65.9	113.4	56.4	0.008
Vitamin D intake (IU)	76.4	48.8	69.3	40.6	66.9	53.7	0.213
Vitamin E intake (mg)	8.4	2.8	8.3	3.2	7.5	2.7	0.021
Salt intake (g)	13.5	4.1	13.7	4.8	12.8	4.5	0.291
Cholesterol intake (mg)	323.7	122.6	322.9	160.2	304.7	137.5	0.412

Analysis of variance was used to examine the differences in the mean values of factors among groups

SD standard deviation

Table 2 Relationship between serum micronutrients and tobacco smoking status

	Non-smoker ($N = 190$)		Past smoker ($N = 56$)		Current smoker ($N = 142$)		P value
	Adjusted mean	95 % CI	Adjusted mean	95 % CI	Adjusted mean	95 % CI	
Serum retinol (μg/dL)	59.23	56.42–62.04	59.70	54.59–64.81	60.88	57.24–64.51	0.695
Serum α-carotene (μg/dL)	9.70	8.58–10.82	7.47	5.43–9.51	7.23	5.78–8.68	0.003
Serum β-carotene (μg/dL)	58.05	50.77–65.33	46.61	33.36–59.85	41.02	31.60–50.44	0.003
Serum zeaxanthin/lutein (μg/dL)	54.93	50.77–59.09	54.06	46.50–61.62	49.88	44.50–55.26	0.205
Serum cryptoxanthin (μg/dL)	31.19	25.61–36.76	23.61	13.46–33.76	21.27	14.05–28.49	0.03
Serum lycopene (μg/dL)	30.00	26.76–33.22	34.68	28.80–40.55	27.23	23.04–31.41	0.06
Serum α-tocopherol (μg/dL)	881.68	817.51–945.84	953.15	836.40–1,069.91	873.56	790.50–956.63	0.414

Analysis of covariance was used to examine the differences in the mean concentrations of the serum levels of micronutrients that are related to the effect of the smoking status. The data were adjusted for age (20–29, 30–39, or 40–54 years), BMI and alcohol intake frequency (0, 1–6, 7/week)

(HR 0.68, 95 % CI 0.49–0.95) as compared with women in the lower tertiles, but the linear trend was not statistically significant ($p = 0.882$). From the questionnaire, high-load intake of retinol significantly inhibited the regression (adjusted model: HR 0.59, 95 % CI 0.40–0.89) but the linear trend was not significant (Table 3).

Because serum levels of most carotenoids were low and carotene intake was small in smokers, the regression group was sub-analyzed stratifying by smoking status (never or current smokers) as shown in Tables 4 and 5. In non-smokers (Table 4), regression was observed in women in the upper tertiles of serum zeaxanthin/lutein (HR 1.25, 95 % CI 0.78–2.01) as compared with women in the lower and middle tertiles, and the linear trend was statistically significant ($p = 0.024$). In current smokers, this was statistically abolished as shown in Table 5. In current smokers, a significant inhibition of regression was observed in women in the middle tertiles for serum alpha-tocopherol (HR 0.53, 95 % CI 0.27–0.94) as compared with women in the lower tertiles, and the linear trend was significant ($p = 0.042$) in the adjusted model (Table 5).

Effect of serum micronutrients and nutrient intake in progression subjects

In Table 6, a significant inverse relationship was observed in subjects with a medium level of serum beta-carotene (HR 0.28, 95 % CI 0.11–0.71, $p = 0.007$), although these

Table 3 HR of regression from entire CIN1/2 according to the serum micronutrients and nutrient intake questionnaire

	n	Person-months	Events	Cumulative 2-year rate (95 % CI)	Hazard ratio for regression (95 % CI)			
					Unadjusted	*p* value	Adjusted model	*p* value
Serum retinol							*p* for trend	0.812
Low (<55.2)	128	1,715.6	74	62.5 (53.6–71.4)	1		1	
Medium (55.2–67.9)	132	1,689.8	77	63.2 (54.4–72.0)	1.06 (0.77–1.46)	0.709	1.19 (0.86–1.65)	0.301
High (>67.9)	131	1,763.5	67	57.8 (48.6–67.4)	0.87 (0.62–1.21)	0.399	0.87 (0.62–1.22)	0.423
Serum α-carotene							*p* for trend	0.472
Low (<5.1)	127	1,654.9	71	60.9 (51.9–70.0)	1.00		1.00	
Medium (5.1–9.7)	133	1,750.0	68	57.3 (48.2–66.8)	0.91 (0.65–1.27)	0.574	1.00 (0.71–1.41)	0.984
High (>9.7)	131	1,764.0	79	65.2 (56.5–73.9)	1.04 (0.75–1.43)	0.828	1.26 (0.89–1.80)	0.19
Serum β-carotene							*p* for trend	0.095
Low (<28.3)	129	1,679.7	66	56.7 (47.7–66.2)	1.00		1.00	
Medium (28.3–57.6)	131	1,755.9	75	62.7 (53.8–71.6)	1.10 (0.79–1.53)	0.581	1.17 (0.83–1.66)	0.364
High (>57.6)	131	1,733.3	77	64.0 (55.2–72.9)	1.12 (0.80–1.56)	0.511	1.34 (0.93–1.93)	0.115
Serum zeaxanthin/lutein							*p* for trend	0.235
Low (<42.9)	130	1,645.9	76	62.7 (53.8–71.6)	1.00		1.00	
Medium (42.9–57.3)	130	1,803.1	70	58.1 (49.2–67.2)	0.85 (0.62–1.18)	0.341	0.97 (0.69–1.36)	0.868
High (>57.3)	131	1,719.9	72	63.5 (54.2–72.7)	0.89 (0.65–1.23)	0.488	1.05 (0.75–1.48)	0.768
Serum cryptoxanthin							*p* for trend	0.215
Low (<11.2)	129	1,659.5	74	63.9 (54.8–73.0)	1.00		1.00	
Medium (11.2–22.1)	130	1,754.7	67	56.8 (47.8–66.2)	0.87 (0.62–1.21)	0.406	0.91 (0.65–1.28)	0.592
High (>22.1)	132	1,754.7	77	63.1 (54.3–71.9)	0.99 (0.72–1.37)	0.974	1.07 (0.76–1.51)	0.694
Serum lycopene							*p* for trend	0.638
Low (<19.8)	129	1,713.7	69	58.6 (49.7–67.9)	1.00		1.00	
Medium (19.8–35.8)	131	1,780.3	79	66.3 (57.4–75.0)	1.07 (0.78–1.48)	0.67	1.07 (0.76–1.49)	0.705
High (>35.8)	131	1,674.9	70	58.5 (49.4–67.8)	1.02 (0.73–1.42)	0.914	1.08 (0.77–1.52)	0.662
Serum α-tocopherol							*p* for trend	0.882
Low (<753.0)	128	1,535.8	82	67.3 (58.7–75.6)	1.00		1.00	
Medium (753.0–983.9)	132	1,896.8	66	54.7 (45.9–64.0)	0.66 (0.48–0.91)	0.011	0.68 (0.49–0.95)	0.025
High (>983.9)	131	1,736.3	70	62.8 (53.2–72.3)	0.74 (0.54–1.01)	0.062	0.78 (0.56–1.09)	0.142
Retinol intake							*p* for trend	0.322
Low (<190.2)	130	1,555.8	74	62.8 (53.6–72.0)	1.00		1.00	
Medium (190.2–313.1)	130	1,755.6	74	63.3 (54.0–72.0)	0.89 (0.65–1.23)	0.484	0.76 (0.54–1.07)	0.12
High (>313.1)	131	1,857.5	70	57.8 (49.0–66.9)	0.80 (0.57–1.10)	0.172	0.59 (0.40–0.89)	0.011
Carotene intake							*p* for trend	0.325
Low (<3,281.4)	130	1,639.3	70	59.8 (50.6–69.1)	1.00		1.00	
Medium (3,281.4–5,042.8)	131	1,812.8	72	61.6 (52.5–64.7)	0.92 (0.66–1.28)	0.637	0.90 (0.63–1.28)	0.557
High (>5,042.8)	130	1,716.8	76	62.2 (53.5–71.0)	1.03 (0.74–1.42)	0.869	0.97 (0.65–1.46)	0.89
Vitamin A intake							*p* for trend	0.546
Low (<2,398.8)	130	1,601.8	70	61.5 (62.5–74.6)	1.00		1.00	
Medium (2,398.8–3,466.7)	131	1,834.7	72	59.7 (51.7–64.7)	0.90 (0.65–1.25)	0.541	0.91 (0.64–1.29)	0.599
High (>3,466.7)	130	1,732.4	76	62.6 (53.9–71.4)	1.01 (0.73–1.40)	0.948	0.93 (0.61–1.42)	0.727
Vitamin E intake							*p* for trend	0.147
Low (<6.7)	130	1,610.2	68	57.4 (48.3–66.7)	1.00		1.00	
Medium (6.7–8.7)	130	1,897.1	71	59.4 (50.5–68.5)	0.90 (0.64–1.25)	0.521	0.95 (0.66–1.39)	0.807
High (>8.7)	131	1,661.6	79	65.9 (57.1–74.6)	1.11 (0.80–1.54)	0.519	0.88 (0.54–1.43)	0.601

Cox's proportional hazard model showing the hazard ratio for regression in a cumulative 24-month period. The adjusted model was calculated by CIN grade (initial biopsy results; CIN1 or CIN2), HPV genotypes (HPV16/18/31/33/35/42/52/59, other high-risk types, low-risk types, or HPV negative) [17, 18], age, total calorie intake and smoking status (Brinkman index >100). The units of micronutrients are expressed as μg/dL