CASE REPORT

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Non-invasive imaging techniques for early diagnosis of radiation-induced squamous cell carcinoma of the lip

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Abstract

Ionizing radiation was used in the past for treatment of several benign cutaneous conditions, and particularly hemangiomas. Even though radiotherapy was generally effective, it significantly augmented the risk for malignancies, resulting in a consequent heavy decline of its use for benign conditions. However, radiation-induced neoplasia is still encountered in adult patients irradiated during childhood or adolescence. We report a case of well-differentiated squamous cell carcinoma developing on the lower lip of a 59-year-old woman who had previously undergone local radiotherapy for a hemangioma, in which the use of non-invasive imaging techniques such as *in vivo* reflectance confocal microscopy (RCM) and dermoscopy allowed an early diagnosis and a prompt, effective treatment. Owing to its capability of assessing microscopic features of dysplasia and neoplastic changes, RCM in combination with dermoscopy may provide an invaluable tool for early detection of malignant changes in previously irradiated cutaneous and mucosal areas.

Keywords: squamous cell carcinoma, hemangioma, lip neoplasms, radiation-induced cancer, *in vivo* reflectance confocal microscopy, dermoscopy.

Introduction

Squamous cell carcinoma (SCC) is the most common form of oral and lip malignancy and accounts for over 90% of all cancers of this area, men being affected more often than women [1–5]. Sun exposure, tobacco consumption, high alcohol intake, chronic inflammatory processes, viral infections, and immunosuppression are all risk factors that have been related to lip neoplasms [4, 6–8]. Nowadays, it is well documented that childhood radiotherapy is associated with the occurrence of benign and malignant tumors, and the association between SCC development and skin exposure to X-ray irradiation has been known for decades [9]. However, between the 1940s and 1960s many benign cutaneous conditions, and particularly hemangioma, were treated using ionizing radiation [10, 11]. Most patients that underwent radiotherapy for head and neck hemangiomas were children. Even though these treatments were generally effective, they substantially contributed to enhanced risks for malignancy development. In light of the increased awareness of radiation-induced malignancies, radiation therapy for benign diseases is now avoided and extended protection measures against radiation have become a standard in clinical practice. Even so, radiation-induced neoplasia is still seen today in adult patients irradiated during childhood or adolescence.

SCCs developing at the site of ionizing radiation exposure usually overlay areas of chronic radiodermatitis and generally occur many years after multiple radiation sessions with a total dosage greater than 1000 rads [12].

Due to the sometimes-subtle clinical changes in areas of chronic radiodermatitis that may be linked to the development of a SCC, quick, reliable, non-invasive and repetitive diagnostic tools need to be employed for the close monitoring of these patients. Recent studies aim to discover new tumor-related biomarkers, which can be used for early detection, follow-up, and development of new-targeted therapeutic agents for SCC and other mucocutaneous malignancies [13–17].

Over the last years, an increasing interest has been shown for *in vivo* imaging techniques that can be used to evaluate skin and oral mucosa. Dermoscopy has become an essential tool for the investigation of skin tumors. Reflectance confocal microscopy (RCM) is a newer, non-invasive, imaging technology capable of generating horizontal black and white images of epithelial tissue at resolutions approaching those of optical microscopy that can be useful in increasing the diagnostic accuracy in various skin lesions [18–26].

Here we report the case of a patient who developed a SCC of the lower lip, for which dermoscopy and RCM examination allowed an increased diagnostic accuracy and enabled optimal therapy. Considering that timely detection and swift, effective treatment are still the greatest determining factors of long-term survival and quality of life of these patients [27], through this case report we aim to emphasize the potential role of dermoscopy and RCM in the early diagnosis of radiation-induced SCC of the lip.

Case presentation

In April 2016, E. M., a 59-year-old woman was admitted to the Department of Oral and Maxillofacial Surgery, "Dr. Carol Davila" Central Military Emergency Hospital, Bucharest, and was referred to the Department of Dermatology, "Prof. Dr. Nicolae C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania, for evaluation of a growing tumor on her lower lip. The study was conducted in accordance with the guidelines approved by the local Ethics Committee and after the informed consent was obtained from the patient. The patient reported having undergone local radiotherapy for a hemangioma during her childhood. She had annual sessions of radiotherapy for four years, from the age of six until she was 10 years old. Further, since 1996, she underwent argon laser therapy for a period of two years. Clinical examination discovered an increased lower lip volume, atrophic lip surface and a round to oval tumor with a keratotic surface, measuring approximately 10 mm in diameter (Figure 1A). Differential diagnosis based on clinical examination included SCC, followed by an array of other cutaneous tumors, soft tissue tumors, vascular tumors, pyogenic granuloma, *dermatofibrosarcoma protuberans* and amelanotic melanoma.

Dermoscopy, using a digital videodermoscopy system (FotoFinder, Teachscreen, Germany) and the VivaScope 1500 VivaCam macro camera (Lucid Inc., Rochester, NY, USA) revealed a white-yellowish astructural area surrounded by telangiectatic, tortuous vessels (Figure 1B). *In vivo* RCM was performed using a commercially available reflectance confocal microscope (Vivascope 1500 Caliber I.D., NY, USA). RCM examination of the epidermis identified a deeply altered keratinocyte architecture (disarrangement of the spinous and granular layer) and extensive cell atypia (Figure 1C). Dilated, tortuous blood vessels containing refractile elements corresponding to erythrocytes could be identified on basic RCM images (Figure 1D) and inflammatory infiltrates (Figure 1E) could be identified on basic RCM images. A rather particular "swirl" aspect could be seen at the level of the *stratum spinosum* (Figure 1F), possibly corresponding to keratin pearls caused by intraepithelial keratinization.

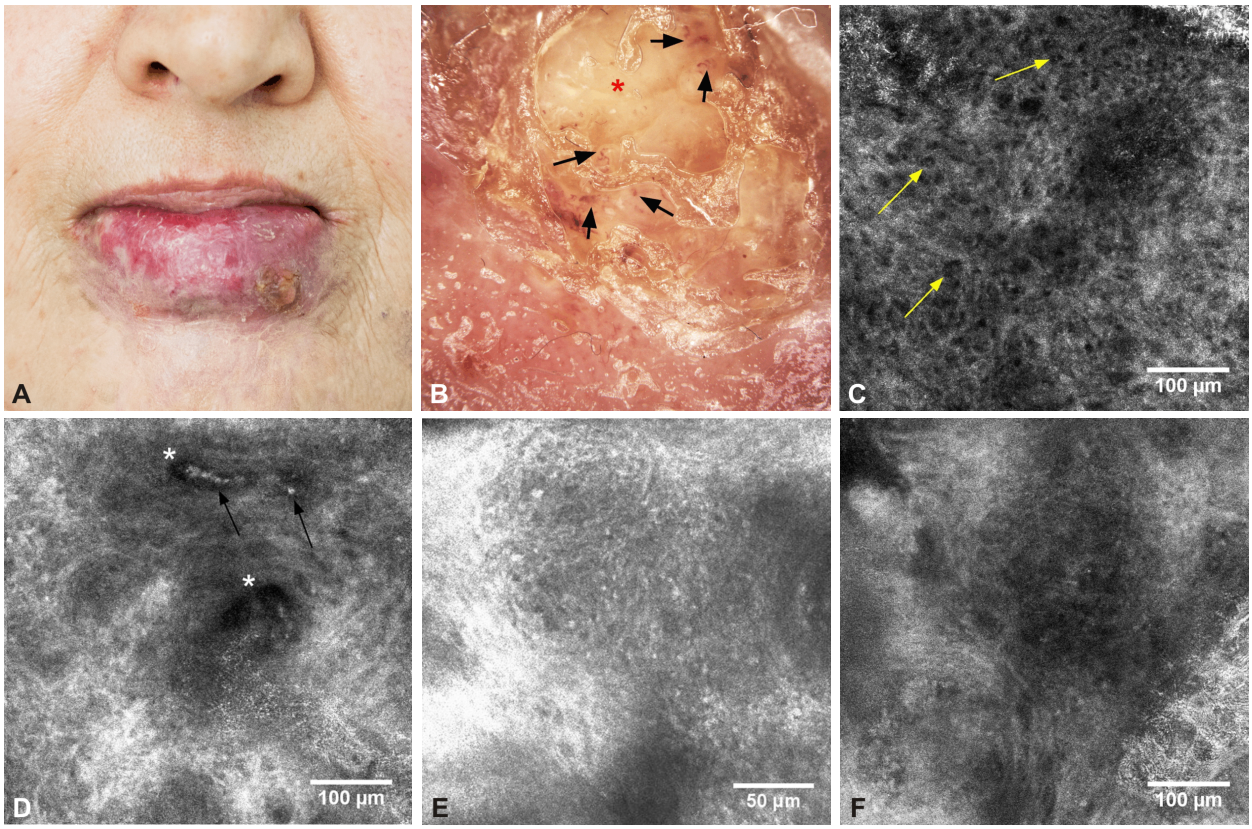

Figure 1 – ***(A) Clinical image illustrating an increased lower lip volume, and a round to oval tumor with a keratotic surface on a background of lower lip surface atrophy; (B) Dermoscopy image showing a white-yellowish astructural area (red asterisk) surrounded by telangiectatic tortuous vessels (black arrows); (C) RCM image at the stratum spinosum showing deeply altered keratinocyte architecture (disarrangement of the spinous layer) and pleomorphic keratinocytes (yellow arrows); (D) RCM image showing dilated, tortuous vessels (white asterisk) containing refractile elements corresponding to erythrocytes (black arrows); (E) RCM image depicting bright elements corresponding to inflammatory cells; (F) Basic RCM image showing the distinct "swirl" aspect (lower left corner of the image) at the level of the stratum spinosum, possibly corresponding to keratin pearls caused by intraepithelial keratinization. RCM: Reflectance confocal microscopy.***

Histopathological examination was performed after standard Hematoxylin and Eosin staining of the excised tissue and confirmed the diagnosis of invasive, well differentiated, SCC. Tumor proliferation did not however infiltrate the underlying muscular fibers and an important chronic inflammatory infiltrate consisting of lymphocytes and plasmocytes was present (Figure 1, G and H). Treatment consisted of wedge-excision vermilionectomy (Figure 1I) in the Department of Oral and Maxillofacial Surgery, "Dr. Carol Davila" Central Military Emergency Hospital, Bucharest, with good cosmetic and functional results nine months after surgery (Figure 1J).

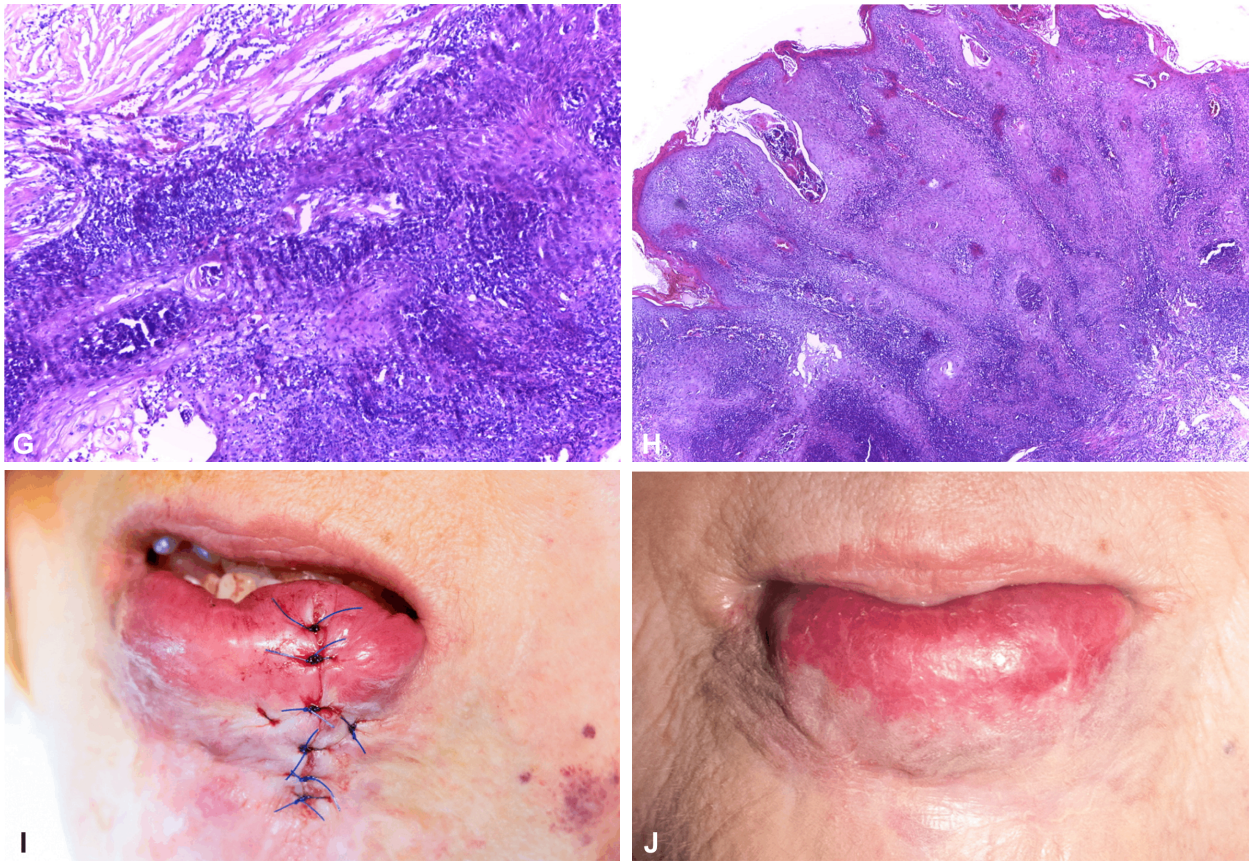

Figure 1 (continued) – ***(G and H) Microscopy images (Hematoxylin and Eosin staining, ×10 and ×4 magnification, respectively) confirming the diagnosis of invasive well differentiated squamous cell carcinoma without muscular invasion – a chronic inflammatory infiltrate consisting of lymphocytes and plasmocytes can be seen; (I) Clinical image of the patient immediately after wedge-excision vermilionectomy; (J) Clinical image nine months after surgery showing good cosmetic results and no sign of recurrence.***

Discussions

Radiation-induced solid tumors, such as SCC, have a long latent period (10 years or longer), and their incidence is directly proportional to the total dose of radiation and the time passed since exposure [28–32]. The latent period is inversely proportional to the radiation dose; the larger the dosage, the longer the latent period before tumor development [33]. It would appear that the age at the time of irradiation may also affect the latent period; the older the age at exposure, the shorter the latent period [34]. A study on radiation-induced malignancy [35] has estimated the radiation-related cancer rate in exposed tissues to be 1.8 cases/million person-year/rad. Other authors report the dose-effect relationship between epithelial cancers and radiation exposed skin as 40 carcinomas/104 persons/Gy over a median follow-up period of 41 years [28].

Importantly, children are significantly more susceptible to the carcinogenic effects of radiation therapy than adults; children also have a longer life expectancy, leading to a bigger window of opportunity for these effects to manifest themselves.

According to early epidemiological studies, most radiation-induced skin cancers are basal cell carcinomas, with only a small percentage being represented by SCC [28]. A newer population-based study from Canada [36] found a 5.7- and 4.8-fold increase in the incidence of BCC and SCC, respectively, associated with non-diagnostic ionizing radiation exposure. Karagas *et al.* [37] reported a relative risk of 1.7 and 1.0 for new BCC and SCC radiation-related occurrences, respectively.

In SCC patients, early detection allowing a prompt, effective treatment is one of the most important factors for long-term survival and quality of life. Hence, modern imaging techniques such as RCM and dermoscopy are more and more present in the investigation of muco-cutaneous tumors.

RCM enables the *in vivo* assessment of cellular and sub-cellular skin morphology down to a depth of approximately 300 μm. Over the recent years, RCM has successfully been employed in the evaluation of neoplastic, inflammatory, and infectious skin disorders alike. Various studies have described the confocal microscopy features of basal cell carcinoma [20, 21, 26, 38, 39], actinic keratosis and cutaneous SCC [40–43], benign melanocytic nevi and melanoma [44–47], inflammatory skin conditions [48, 49],

such as psoriasis [22, 50, 51] and *pityriasis rubra pilaris* [52], vesiculobullous disorders [53], and infectious diseases [54–58].

The biggest advantage of RCM over classical diagnostic approaches is its noninvasive nature. RCM allows for fast, real time evaluation of epidermal and partially dermal tissue architecture without patient discomfort and without the need for further equipment or laboratory devices.

RCM criteria for the diagnosis of lip SCC have previously been explored and have been described as extensive keratinocyte pleomorphism creating a disarranged honeycomb pattern of the *stratum spinosum*, round and bright nucleated cells (representing atypical and dyskeratotic keratinocytes), as well as atypical nucleated cells in the superficial dermis in invasive SCCs [43]. Upon RCM examination in our case, we observed a markedly disarranged *stratum spinosum*, as well as extensive keratinocyte atypia, and dilated blood vessels surrounded by perivascular inflammatory infiltrates. The dermis presented thickened collagen bundles as well as inflammatory infiltrates.

The management of radiation-induced solid tumors includes surgery and radiotherapy, taking into account the site and extent of tumor involvement. Considering the now so well known late effects of ionizing radiation exposure, radiotherapy for benign skin lesions is contra-indicated, especially in the view of the body of evidence that proves low doses of radiation applied over long periods of time dramatically increase the risk of cancer development [28, 59].

Owing to the long latent period between radiation exposure and SCC development, non-invasive, reliable imaging technologies, such as dermoscopy and RCM may prove to be invaluable tools in skin cancer diagnosis algorithms, thus adding substantial benefits to patients and clinicians alike. Because of its potential to assess microscopic features of normal mucosa, dysplasia, and neoplastic changes, RCM may also provide a viable alternative for evaluation of mucosal tumor margins during surgery [60], thereby leading to improved surgical times and appreciable cost reductions when compared to frozen section histology.

Conclusions

Patients with a personal history of radiotherapy need lifetime lasting dermatological observation on account of the fact that the management of large, radiation-induced cutaneous tumors can be rather complicated.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' contribution

The first two authors, Mihai Lupu and Ana Căruntu, contributed equally to writing and editing the manuscript.

References

[1] Cooper JS, Porter K, Mallin K, Hoffman HT, Weber RS, Ang KK, Gay EG, Langer CJ. National Cancer Database report on cancer of the head and neck: 10-year update. Head Neck, 2009, 31(6):748–758.

[2] Stucker FJ, Lian TS. Management of cancer of the lip. Oper Tech Otolaryngol Head Neck Surg, 2004, 15(4):226–233.

[3] Papadopoulos O, Konofaos P, Tsantoulas Z, Chrisostomidis C, Frangoulis M, Karakitsos P. Lip defects due to tumor excision: apropos of 899 cases. Oral Oncol, 2007, 43(2):204–212.

[4] Galyon SW, Frodel JL. Lip and perioral defects. Otolaryngol Clin North Am, 2001, 34(3):647–666.

[5] Calcaianu N, Popescu SA, Diveica D, Lascar I. Surgical attitude in premalignant lesions and malignant tumors of the lower lip. J Med Life, 2015, 8(1):109–111.

[6] de Visscher JG, van der Waal I. Etiology of cancer of the lip. A review. Int J Oral Maxillofac Surg, 1998, 27(3):199–203.

[7] Neagu M, Caruntu C, Constantin C, Boda D, Zurac S, Spandidos DA, Tsatsakis AM. Chemically induced skin carcinogenesis: updates in experimental models (Review). Oncol Rep, 2016, 35(5):2516–2528.

[8] Boda D, Neagu M, Constantin C, Voinescu RN, Caruntu C, Zurac S, Spandidos DA, Drakoulis N, Tsoukalas D, Tsatsakis AM. HPV strain distribution in patients with genital warts in a female population sample. Oncol Lett, 2016, 12(3):1779–1782.

[9] Ron E. Cancer risks from medical radiation. Health Phys, 2003, 85(1):47–59.

[10] Lindberg S. Radiotherapy of childhood haemangiomas: from active treatment to radiation risk estimates. Radiat Environ Biophys, 2001, 40(3):179–189.

[11] Lindberg S, Karlsson P, Arvidsson B, Holmberg E, Lundberg LM, Wallgren A. Cancer incidence after radiotherapy for skin haemangioma during infancy. Acta Oncol, 1995, 34(6):735–740.

[12] Brownstein MH, Rabinowitz AD. The precursors of cutaneous squamous cell carcinoma. Int J Dermatol, 1979, 18(1):1–16.

[13] Voiculescu V, Calenic B, Ghita M, Lupu M, Caruntu A, Moraru L, Voiculescu S, Ion A, Greabu M, Ishkitiev N, Caruntu C. From normal skin to squamous cell carcinoma: a quest for novel biomarkers. Dis Markers, 2016, 2016: 4517492.

[14] Ion A, Popa IM, Papagheorghe LML, Lisievici C, Lupu M, Voiculescu V, Caruntu C, Boda D. Proteomic approaches to biomarker discovery in cutaneous T-cell lymphoma. Dis Markers, 2016, 2016:9602472.

[15] Lupu M, Caruntu C, Ghita MA, Voiculescu V, Voiculescu S, Rosca AE, Caruntu A, Moraru L, Popa IM, Calenic B, Greabu M, Costea DE. Gene expression and proteome analysis as sources of biomarkers in basal cell carcinoma. Dis Markers, 2016, 2016:9831237.

[16] Zurac S, Neagu M, Constantin C, Cioplea M, Nedelcu R, Bastian A, Popp C, Nichita L, Andrei R, Tebeica T, Tanase C, Chitu V, Caruntu C, Ghita M, Popescu C, Boda D, Mastalier B, Maru N, Daha C, Andreescu B, Marinescu I, Rebosapca A, Staniceanu F, Negroiu G, Ion DA, Nikitovic D, Tzanakakis GN, Spandidos DA, Tsatsakis AM. Variations in the expression of TIMP1, TIMP2 and TIMP3 in cutaneous melanoma with regression and their possible function as prognostic predictors. Oncol Lett, 2016, 11(5):3354–3360.

[17] Calenic B, Greabu M, Caruntu C, Tanase C, Battino M. Oral keratinocyte stem/progenitor cells: specific markers, molecular signaling pathways and potential uses. Periodontol 2000, 2015, 69(1):68–82.

[18] Rajadhyaksha M, González S, Zavislan JM, Anderson RR, Webb RH. *In vivo* confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. J Invest Dermatol, 1999, 113(3):293–303.

[19] Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. *In vivo* confocal scanning laser microscopy of human skin: melanin provides strong contrast. J Invest Dermatol, 1995, 104(6):946–952.

[20] Ghita MA, Caruntu C, Rosca AE, Kaleshi H, Caruntu A, Moraru L, Docea AO, Zurac S, Boda D, Neagu M, Spandidos DA, Tsatsakis AM. Reflectance confocal microscopy and dermoscopy for *in vivo*, non-invasive skin imaging of superficial basal cell carcinoma. Oncol Lett, 2016, 11(5):3019–3024.

[21] Căruntu C, Boda D, Guţu DE, Căruntu A. *In vivo* reflectance confocal microscopy of basal cell carcinoma with cystic degeneration. Rom J Morphol Embryol, 2014, 55(4):1437–1441.

[22] Căruntu C, Boda D, Căruntu A, Rotaru M, Baderca F, Zurac S. *In vivo* imaging techniques for psoriatic lesions. Rom J Morphol Embryol, 2014, 55(3 Suppl):1191–1196.

[23] Diaconeasa A, Boda D, Neagu M, Constantin C, Căruntu C, Vlădău L, Guţu D. The role of confocal microscopy in the dermato-oncology practice. J Med Life, 2011, 4(1):63–74.

[24] Căruntu C, Boda D. Evaluation through *in vivo* reflectance confocal microscopy of the cutaneous neurogenic inflammatory reaction induced by capsaicin in human subjects. J Biomed Opt, 2012, 17(8):085003.

[25] Ghiţă MA, Căruntu C, Rosca AE, Căruntu A, Moraru L, Constantin C, Neagu M, Boda D. Real-time investigation of skin blood flow changes induced by topical capsaicin. Acta Dermatovenerol Croat, 2017, 25(3):223–227.
[26] Lupu M, Căruntu C, Solomon I, Popa A, Lisievici C, Drăghici C, Papagheorghe L, Voiculescu VM, Giurcăneanu C. The use of *in vivo* reflectance confocal microscopy and dermoscopy in the preoperative determination of basal cell carcinoma histopathological subtypes. DermatoVenerol (Bucharest), 2017, 62(4):265–275.
[27] Ridgway JM, Armstrong WB, Guo S, Mahmood U, Su J, Jackson RP, Shibuya T, Crumley RL, Gu M, Chen Z, Wong BJF. *In vivo* optical coherence tomography of the human oral cavity and oropharynx. Arch Otolaryngol Head Neck Surg, 2006, 132(10):1074–1081.
[28] van Vloten WA, Hermans J, van Daal WAJ. Radiation-induced skin cancer and radiodermatitis of the head and neck. Cancer, 1987, 59(3):411–414.
[29] Martin H, Strong E, Spiro RH. Radiation-induced skin cancer of the head and neck. Cancer, 1970, 25(1):61–71.
[30] Hempelmann LH, Hall WJ, Phillips M, Cooper RA, Ames WR. Neoplasms in persons treated with X-rays in infancy: fourth survey in 20 years. J Natl Cancer Inst, 1975, 55(3):519–530.
[31] Hutchison GB. Late neoplastic changes following medical irradiation. Cancer, 1976, 37(S2):1102–1107.
[32] Shore RE, Albert RE, Pasternack BS. Follow-up study of patients treated by X-ray epilation for *Tinea capitis*; resurvey of post-treatment illness and mortality experience. Arch Environ Health, 1976, 31(1):21–28.
[33] Kim JH, Chu FC, Woodard HQ, Melamed MR, Huvos A, Cantin J. Radiation-induced soft-tissue and bone sarcoma. Radiology, 1978, 129(2):501–508.
[34] Nishimura Y, Sakai K, Kitamura T, Hinata H, Yamashita H. [Radiation-induced cancers following radiotherapy of benign diseases: the second mail survey in Japan]. Gan To Kagaku Ryoho, 1986, 13(4 Pt 2):1492–1498.
[35] Li FP, Cassady JR, Jaffe N. Risk of second tumors in survivors of childhood cancer. Cancer, 1975, 35(4):1230–1235.
[36] Gallagher RP, Bajdik CD, Fincham S, Hill GB, Keefe AR, Coldman A, McLean DI. Chemical exposures, medical history, and risk of squamous and basal cell carcinoma of the skin. Cancer Epidemiol Biomarkers Prev, 1996, 5(6):419–424.
[37] Karagas MR, McDonald JA, Greendberg ER, Stukel TA, Weiss JE, Baron JA, Stevens MM. Risk of basal cell and squamous cell skin cancers after ionizing radiation therapy. For The Skin Cancer Prevention Study Group. J Natl Cancer Inst, 1996, 88(24):1848–1853.
[38] González S, Tannous Z. Real-time, *in vivo* confocal reflectance microscopy of basal cell carcinoma. J Am Acad Dermatol, 2002, 47(6):869–874.
[39] Peppelman M, Wolberink EAW, Blokx WAM, van de Kerkhof PCM, van Erp PEJ, Gerritsen MJP. *In vivo* diagnosis of basal cell carcinoma subtype by reflectance confocal microscopy. Dermatology, 2013, 227(3):255–262.
[40] Ulrich M, Zalaudek I, Welzel J. Shining into the white: the spectrum of epithelial tumors from actinic keratosis to squamous cell carcinoma. Dermatol Clin, 2016, 34(4):459–467.
[41] Aghassi D, Anderson RR, González S. Confocal laser microscopic imaging of actinic keratoses *in vivo*: a preliminary report. J Am Acad Dermatol, 2000, 43(1 Pt 1):42–48.
[42] Ulrich M, Maltusch A, Röwert-Huber J, González S, Sterry W, Stockfleth E, Astner S. Actinic keratoses: non-invasive diagnosis for field cancerisation. Br J Dermatol, 2007, 156(Suppl 3):13–17.
[43] Rishpon A, Kim N, Scope A, Porges L, Oliviero MC, Braun RP, Marghoob AA, Fox CA, Rabinovitz HS. Reflectance confocal microscopy criteria for squamous cell carcinomas and actinic keratoses. Arch Dermatol, 2009, 145(7):766–772.
[44] Pellacani G, Guitera P, Longo C, Avramidis M, Seidenari S, Menzies S. The impact of *in vivo* reflectance confocal microscopy for the diagnostic accuracy of melanoma and equivocal melanocytic lesions. J Invest Dermatol, 2007, 127(12):2759–2765.
[45] Ali FR, Craythorne EE. Integration of reflectance confocal microscopy into clinical practice for the management of *lentigo maligna*. Clin Exp Dermatol, 2017, 42(5):593–595.
[46] Agozzino M, Ferrari A, Cota C, Franceschini C, Buccini P, Eibenshutz L, Ardigò M. Reflectance confocal microscopy analysis of equivocal melanocytic lesions with severe regression. Skin Res Technol, 2018, 24(1):9–15.
[47] Lovatto L, Carrera C, Salerni G, Alós L, Malvehy J, Puig S. *In vivo* reflectance confocal microscopy of equivocal melanocytic lesions detected by digital dermoscopy follow-up. J Eur Acad Dermatol Venereol, 2015, 29(10):1918–1925.
[48] Białek-Galas K, Wielowieyska-Szybińska D, Dyduch G, Wojas-Pelc A. The use of reflectance confocal microscopy in selected inflammatory skin diseases. Pol J Pathol, 2015, 66(2):103–108.
[49] Agozzino M, González S, Ardigò M. Reflectance confocal microscopy for inflammatory skin diseases. Actas Dermosifiliogr, 2016, 107(8):631–639.
[50] Ardigo M, Cota C, Berardesca E, González S. Concordance between *in vivo* reflectance confocal microscopy and histology in the evaluation of plaque psoriasis. J Eur Acad Dermatol Venereol, 2009, 23(6):660–667.
[51] Batani A, Brănişteanu DE, Ilie MA, Boda D, Ianosi S, Ianosi G, Caruntu C. Assessment of dermal papillary and microvascular parameters in *psoriasis vulgaris* using *in vivo* reflectance confocal microscopy. Exp Ther Med, 2018, 15(2):1241–1246.
[52] Paganelli A, Ciardo S, Odorici G, Pellacani G, Conti A. Efficacy of ustekinumab after failure of infliximab CT-P13 in a HLA-Cw6-positive patient affected by *pityriasis rubra pilaris*: monitoring with reflectance confocal microscopy (RCM) and optical coherence tomography (OCT). J Eur Acad Dermatol Venereol, 2016, 31(5):e249–e251.
[53] Lacarrubba F, Verzì AE, Pippione M, Micali G. Reflectance confocal microscopy in the diagnosis of vesicobullous disorders: case series with pathologic and cytologic correlation and literature review. Skin Res Technol, 2016, 22(4):479–486.
[54] Lacarrubba F, Verzì AE, Musumeci ML, Micali G. Early diagnosis of herpes zoster by handheld reflectance confocal microscopy. J Am Acad Dermatol, 2015, 73(6):e201–e203.
[55] Lacarrubba F, Verzì AE, Ardigò M, Micali G. Handheld reflectance confocal microscopy for the diagnosis of *molluscum contagiosum*: histopathology and dermoscopy correlation. Australas J Dermatol, 2017, 58(3):e123–e125.
[56] Cinotti E, Labeille B, Cambazard F, Biron AC, Chol C, Leclerq A, Jaffelin C, Perrot JL. Videodermoscopy compared to reflectance confocal microscopy for the diagnosis of scabies. J Eur Acad Dermatol Venereol, 2016, 30(9):1573–1577.
[57] Buljan M, Zalaudek I, Massone C, Hofmann-Wellenhof R, Fink-Puches R, Arzberger E. Dermoscopy and reflectance confocal microscopy in cutaneous leishmaniasis on the face. Australas J Dermatol, 2016, 57(4):316–318.
[58] Pharaon M, Gari-Toussaint M, Khemis A, Zorzi K, Petit L, Martel P, Baran R, Ortonne JP, Passeron T, Lacour JP, Bahadoran P. Diagnosis and treatment monitoring of toenail onychomycosis by reflectance confocal microscopy: prospective cohort study in 58 patients. J Am Acad Dermatol, 2014, 71(1):56–61.
[59] Shibuya H, Takagi M, Kitagawa M, Shioiri S. Squamous cell carcinoma of the oral cavity after irradiation for nonmalignant lesions: report of four cases. J Oral Maxillofac Surg, 1992, 50(1):66–71.
[60] Clark AL, Gillenwater AM, Collier TG, Alizadeh-Naderi R, El-Naggar AK, Richards-Kortum RR. Confocal microscopy for real-time detection of oral cavity neoplasia. Clin Cancer Res, 2003, 9(13):4714–4721.

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