THE USE OF LIQUID-BASED CYTOLOGY IN DIAGNOSIS OF BASAL CELL CARCINOMA AND ACTINIC KERATOSIS

The CYT-BAK study

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ABSTRACT

Background: Ahead of new non-invasive treatments of non-melanoma skin cancer, cytology has proven beneficial and may become the diagnostic method of choice as it results in little tissue disfiguration and thus a superior cosmetic outcome at the sampling site compared to biopsy. Liquid-based cytology (LBC) is fast, easy, and inexpensive in use and may be more beneficial than conventional cytology as it provides instant fixation and preservations of collected cells.

Aims: To evaluate the quality of LBC with the use of ThinPrep®Pap test as a diagnostic tool of BCC and AK and to compare the diagnostic performance of LBC with histopathology.

Methods: This is a prospective, blinded, single-centre pilot study approved by the Regional Committee for Medical Research Ethics. It was performed at the Department of Dermatology and Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim University Hospital. Patients with primary, histologically verified BCC and AK who met the inclusion criteria were recruited to the study during PDT-treatment at the outpatient clinic from September through December 2015. After initial light curettage, a Medscand®Cytobrush was used to collect cells from the tumours. The cells were instantly fixated by rapid transfer to the ThinPrep® Pap test container. The cytological specimens were evaluated by a pathologist and cytologist without knowledge of the histology diagnoses and classified as BCC, AK, other diagnosis or non-evaluable. Cytodiagnostic results were compared with the diagnosis in the histopathology report (the gold standard).

Results: A total of 13 lesions (8 BCC, 5 AK) were included of which 5 samples were classified as non-evaluable. Cytodiagnosis agreed with histopathology in 6 of the 8 BCC cases, and in 2 of the 5 AK cases. Sensitivity and specificity of LBC for diagnosis of BCC was 75% and 100% (95% CI: 0.35-0.97 and 0.48-1.00), respectively. Sensitivity and specificity for diagnosis of AK was 40% and 100% (95% CI: 0.05-0.85 and 0.63-1.00), respectively.

Conclusion: The results suggest that LBC using ThinPrep®Pap test and Medscand®Cytobrush following curettage has a too low sensitivity for routine diagnostic use for diagnosis of BCC and AK. However, when the cell material was representative a correct diagnosis was made in all cases.

PREFACE

The study is part of a student thesis from The Faculty of Medicine, Norwegian University of Science and Technology (NTNU), written by 5th year medical student Sunniva Eriksen Malone Isham. The thesis is within the field of dermatology, with special interest in diagnostics of non-melanoma skin cancer. Primary teaching supervisor is Eidi Christensen, first amanuensis/chief physician, Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU) and Department of Dermatology, St. Olavs Hospital, Trondheim University Hospital. Additional supervisors are Christina Vogt, consultant pathologist, the Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim University Hospital and Department of Laboratory Medicine, Children and Womens Health, Faculty of Medicine, Norwegian University of Science and Technology (NTNU) and Maj-Liv Eide, manager, Section of Cytology at the Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim University Hospital. The motivation for choosing this topic and study is my interest in and desire to learn more about dermatology. The choice of topic is highly relevant as non-melanoma skin cancer is a growing concern in society and something most individuals have a relation to, either directly with self-experienced skin manifestations or indirectly through family-members and friends. Exposure to sunlight through recreational activities and exotic worldwide travels and fashion idols with flawless sun-kissed skin, represents the dangers and a contradictory picture to the physiology of the skin. Excessive exposure to sunlight will damage and alter the skin in time, although the instant beauty and exclusive feel of a perfect tan somehow camouflages the dangers and long-term consequences. The skin is our biggest and most visible organ. It does not usually host the most life-threatening diseases or conditions for sure, but it does however represent a big part of our presentation as humans as it is the armour that houses the life-compatible processes and functional units that in total embraces the miracles of the human body. Diseases of the skin may cause a big psychological impact to the patients affected because of its visibility and handling of such diseases with respect to cosmetic outcome is therefore favourable. The use of liquid-based cytology in diagnostics of non-melanoma skin cancer represents a desire to handle skin damage in a manner that leads to as little alteration of the natural skin structure as possible.

The study was approved by the Regional Committee for Medical Research Ethics (REK 2015/621) on May 20th 2015 (Appendix 1). Cytological patient samples were collected at the Department of Dermatology from September until December 2015. Due to unexpected happenings, the initial clinical work-process was delayed. Our goal was to start the process of sample-collection already in May, but this was unfortunately postponed until September. Therefore, the number of samples collected during my thesis-semester was not as many as we expected. Our original expectation was to collect 20 cytological samples evenly divided between actinic keratosis and basal cell carcinoma. Although this seemed promising prior to start, we found that inclusion of patients into the study was harder than we first thought as many of the patients did not meet the inclusion criteria.

Despite minor challenges on the way, I have learned a lot form the work-process of this thesis and I am grateful to my supervisors for letting me take part in this exciting study. I have learnt that research takes a lot of time and that patience is an important key to success. Nevertheless, time-consuming work becomes

less bothersome when working in a positive, stimulating environment and with a topic of interest. Through reading relevant literature on the field, participating in the photodynamic treatment routines, taking part in cytological evaluation of patient samples and working on this thesis, I have definitely gained a lot of useful knowledge on handling of non-melanoma skin cancer, as well as the use of cytology in diagnostics. Moreover, this opportunity has strengthened my desire to learn more within the field of dermatology.

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Last, but not least, a big thank you to all patients willing to participate in this study.

ABBREVIATIONS

ALA	aminolevulinic acid
AK	actinic keratosis
BCC	basal cell carcinoma
ED & C	electrodissection and curettage
FNAC	fine-needle aspiration cytology
H&E	Haematoxylin & Eosin
LBC	liquid-based cytology
MAL	methyl aminolaevulinate
mBCC	morfea type basal cell carcinoma
MGG	May-Günwald-Giemsa
MM	malignant melanoma
nBCC	nodular type basal cell carcinoma
NMSC	non-melanoma skin cancer
NPE	norsk pasientskadeerstatning
NTNU	Norwegian University of Science and Technology
Pap	papanicolau
PDT	photodynamic therapy
PTCH	protein patched homolog, receptor for sonic hedgehog
RT	radiotherapy
sBCC	superficial type basal cell carcinoma
SCC	squamous cell carcinoma
UV-R	ultraviolet radiation

INTRODUCTION

Cytology is widely used in diagnostics of different types of cancer such as lung cancer (1), bladder cancer (2), thyroid cancer (3) and cervical cancer (4). The use of cytology in diagnosing skin cancer is however not commonly used (5). Biopsy, followed by histopathological evaluation, is considered the gold standard when deciding the final diagnosis of lesions suspicious of skin cancer (5, 6).

With new non-invasive treatment options emerging, such as photodynamic therapy (PDT) and topical creams, it is both desirable and reasonable to combine these with non-invasive diagnostic modalities to create a total non-invasive procedure in the process of diagnostics and treatment. The overall goals are to secure a good cosmetic outcome for the patient and a more efficient and cost-effective technique without the need of anaesthesia and advanced equipment in clinical practice (5-7). Our interest and contribution on the matter is to evaluate the use of liquid-based cytology (LBC) in diagnostis of basal cell carcinoma (BCC) and actinic keratosis (AK). LBC is widely used in screening for cervical cancer. Its use results in higher sensitivity rates compared to the use of conventional fixation and staining methods. LBC is also more effective, easier to perform and allows direct fixation of the harvested cells. Direct fixation is of great advantage as it results in better preservation of the cells (8, 9).

A search through PubMed, EMBASE and Cochrane Library reveals that several studies on the field are available (5-7, 10-21) (Figure 1). Cytology with the use of skin scrape-cytology in diagnosis of BCC and AK has been found to achieve test results with both high sensitivity and specificity (5, 6, 13). The interest in cytology is noticeable and recent studies have tested different techniques within cytodiagnostics, both fine-needle aspiration cytology (FNAC), imprint cytology, exfoliative cytology and skin-scrape cytology. Although all studies show promising results, greater and additional studies are requested to determine whether cytology is as useful a method compared to histology and in which cases cytology can be used in preference to histology (5-7, 10-21). Different preparation techniques are available to make cytological smears. In the earlier mentioned studies, conventional cytopreparatory methods, such as Papanicolaou (Pap)-smears, are frequently used. We have not yet been able to find reports on using LBC in combination with skin-scrape sampling techniques in the investigation of non-melanoma skin cancer (NMSC). As LBC allows immediate fixation of the cells collected, we believe the technique will produce representative smears for diagnostic evaluation. Two recent studies by *Morrison et.al* (15) and *Onuma et.al* (20) evaluated the use of LBC in diagnostics of malignant melanoma(MM) and pemphigus vulgaris, respectively. Both found that LBC is favourable in use and raises the diagnostic quality of smears compared to conventional cytology (15, 20).

Our hypothesis is that LBC can provide a satisfactory amount of representative samples for diagnostic evaluation and also that LBC is a good method for diagnosis of AK and BCC compared to histology.

Therefore, the overall aims of this study were to evaluate the quality of LBC with the use of ThinPrep®Pap test as a diagnostic tool in BCC and AK and also to compare the diagnostic performance of LBC with histopathology.

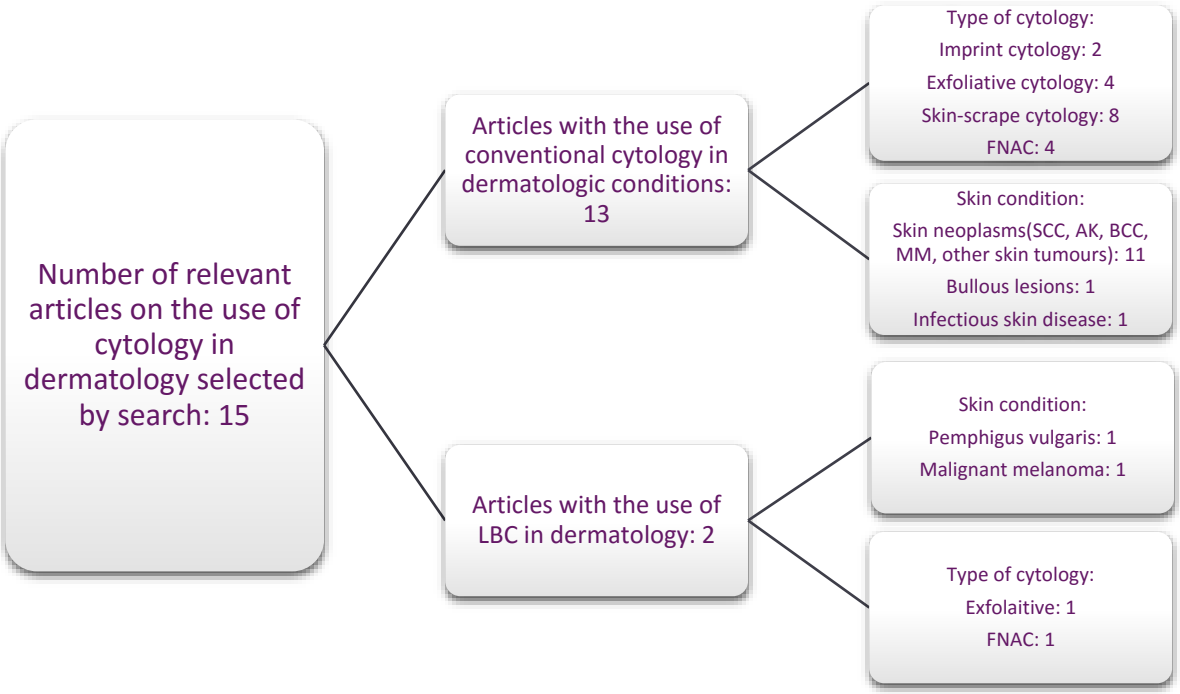


Figure 1. Flow-chart showing selection of relevant articles.

BACKGROUND

Cytology as method

Cytology involves the art and science of the interpretation of cells from the human body (22). Cytology allows visualisation of cell morphology and in diagnostic cytopathology cells are collected from clinically suspect lesions to look at changes in cell morphology by light microscopy. Cytologists are trained to look at cells and to distinguish normal cells from abnormal cells. It is therefore possible to use knowledge of cell morphology to detect pathological processes. Characteristic cell changes and type of cells present are associated with particular medical conditions (23). The use of cytology within the field of medical diagnostics is expanding. Today cells can be collected from nearly all sites and organs of the body. In order to retain useful information from these samples, knowledge regarding fixation techniques, colouring and systematic interpretation of the visualised cells are essential. Alongside the advances in sampling, new fixation and colouring techniques have emerged, and trained and qualified practitioners are responsible for handling the cytological samples which in addition raises the quality in diagnostic cytology. In summary four main steps are important in cytology to ensure an accurate diagnostic quality: sampling technique, fixation method, colouring method and experienced practitioners handling the cell samples. Satisfactory pre-sampling preparation of the requested area and accessibility to the area are of course also factors of importance.

Sampling

Diagnostic cytopathology is based on a few basic sampling techniques (22, 23):

- Collection of exfoliated cells, meaning cells that are naturally shed from the organ because of cyclic renewal of cells.
- Cells removed by brushing or abrasive techniques(scalpel or curette), meaning that not only naturally shed cells are removed, but also cells from the deeper layers are collected and made accessible for visualisation and evaluation. Cells are obtained directly from the requested lesion or organ.
- Aspiration cytology where a needle is guided to the target area by the use of different imaging modalities and cells are collected with the help of negative pressure. The main objective of aspirational biopsy is to distinguish benign from malignant lesions and to classify neoplasms and other pathologic processes.
- Intraoperative cytology with the use of imprint-cytology, meaning that the surgeon is offered intraoperative cytological consultation. Smears are prepared by forcefully pressing clean glass to the surface of the tissue, by scraping or crushing small fragments of tissue between two slides.
- Other methods to mention are lavage and body fluid collection. In lavage cells are mechanically removed from their setting by instillation of saline fluid or other fluid to detach cells from their environment and collect them. In body fluid collection, fluids from body cavities are collected and evaluated to identify conditions such as infectious processes or cancer.

Fixation

Rapid fixation of the cell sample is necessary to preserve cytological detail of cells as air-drying distorts the cells and makes them difficult to recognise. A number of fixation methods exist and are chosen carefully in consideration of desired visualisation method and type of cell sample (Table 1, 22, 24). Different fixation methods produce a variety of cytological smears. The fixation method evaluated in this study is a liquid-based technique which was first used in gynaecology and is a fairly new method. It was developed to ensure enhanced sensitivity and specificity of the Pap smear. The advantage of this method is that cells collected are transferred directly to a container with a liquid-based solution thus enabling instant fixation. In conventional fixation methods cells have to be spread onto a glass slide before they can be fixated (22).

Staining

Numerous techniques for staining are available (Table 2, 22, 24). The Pap stain is highly recommended for use in routine diagnostic cytology. The use of Pap produces smears showing well-stained nuclear chromatin, differential cytoplasmic counterstaining and cytoplasmic transparency (22). It is also an attractive method for differentiating between keratinizing and non-keratinizing cells (6). The cells evaluated in this study were stained using a modified Papanicolaou method. The ability to distinguish other epidermal cells from keratinizing cells is of great importance as the non-keratinizing cells are nucleated and have cytoplasm. These are important in cytological evaluation for obtaining information about pathological processes associated with BCC and AK and to differentiate between malignant and benign cells.

Table 1. Examples of different types of fixation procedures.

Type of fixation	Examples of agents	Recommendation for use	Purpose
Wet fixation: freshly prepared smears are submerged immediately in a liquid fixative.	95% ethanol Ether alcohol mixture 100% methanol 80% propanol and isopropanol	95% ethanol is the ideal fixative recommended in most cases of fixation. Ether alcohol mixture is not frequently used due to safety hazards. 100% methanol is an acceptable substitute for 95% methanol, but is more expensive. 80% propanol and isopropanol is also an acceptable substitute for 95% ethanol.	Optimal cell shrinkage for visualisation of nucleus and appearance of optimal chromatin detail.
Coating fixative: substitutes for wet fixation. Either aerosols applied by spraying or a liquid base dropped onto the slide. Composed of an alcohol and wax-like substance.	Carbowax Diaphine	Poorer quality than wet fixation. Practical for use on samples that need to be transported/sent longer distances to a laboratory. Aerosols are not recommended for bloody smears as they cause clumping of erythrocytes.	Alcohol provides fixation of cells whilst the wax-like substance form a thin protective coating over the smear.
Special purpose fixative	Carnoy's fixative (10% acetic acid) AAF fixative	Carnoy's is a fixative for use on haemorrhagic samples. AAF is ideal for cell block preparation of fluid specimens.	Carnoy's haemolyses red blood cells.
Prefixation: preservation of specimens for days without deterioration of cells.	Ethyl alcohol (50%) Sacomanno's fixative (50% alcohol and 2% carbowax 1540) Mucolexx	Practical for use when specimens cannot be fixated immediately. May result in coagulation of proteins, hardening of cells and condensation of chromatin. May also cause clustering of cells making stain absorption and interpretation difficult.	Preservation of cells over a longer period.

Table 2. Presentation of the most common staining methods

Types of staining	Purpose of use	Result
Papanicolua(Pap)	The routine staining procedure used in cytopathology. The method of choice in gynaecological samples.	Well stained nuclear chromatin, differential cytoplasmic counterstaining and cytoplasmic transparency.
Haematoxylin & Eosin(H&E)	May be used routinely for staining of non-gynaecological smears. Unacceptable for use on cervical smears. Mainly used on histologic sections or aspiration smears.	Provides clear definition of nuclear details and differential counterstaining giving cytoplasmic transparency.
May-Günwald-Giemsa(MGG) E.g Romanowsky	Used for cytological diagnosis of non-gynaecological samples in addition to Pap and H&E. Widely used for staining of air-dried smears, especially blood and bone marrow. Preferred by some for use on all cytological material.	Nucleus stains blue, cytoplasm stains pink.

The use of cytology in dermatology

Normal skin structure

Histologically the skin can be divided into three main layers; the epidermis, the dermis and the subcutis (25). The epidermis is the outermost layer of the skin and consists of a squamous stratified epithelium (keratinocytes) and dendritic cells such as Langerhans cells (immune cells) and melanocytes (pigmented cells). The dermis consists of fibrocollagenous tissue and elastic tissue which contains blood vessels, nerves and sensory receptors. The deepest layer of the skin, the subcutis, consists mainly of adipose tissue, but larger vessels to supply and drain the dermal blood vasculature, are also present. In addition, there are the skin appendages, specialised structures that mainly occupy the dermis, but can also be found in the upper subcutis occasionally. Examples of skin appendages are sweat glands, hair follicles and sebaceous glands. The stratified organisation of the epithelium arranges the cells in four main layers (stratum) due to different cell characteristics and level of differentiation (Table 3, 26). The most superficial keratinocytes are anucleated keratinizing cells. Under normal conditions only keratinizing cells exfoliate. In pathological processes of the skin, normal organisation and differentiation of cells are altered and different types of cells may exfoliate (25).

Table 3. Stratification of the epidermis.

Stratum	Type of cells/function
Stratum basale (the basal layer)	Cuboidal or low columnar cells that connect to the basal membrane below and the stratum spinosum above. Responsible for constant regeneration of cells by repeated mitotic division.
Stratum spinosum (the prickle cell layer)	Composed of polyhedral keratinocytes with large and prominent nucleoli. Active protein synthesis function which generates fibrillary proteins that form desmosomes. Desmosomes create connecting bridges between adjacent keratinocytes
Stratum granulosum (the granular layer)	Loss of polyhedral shape, more squamous-like cells. The cells contain protein-rich granules for production of keratin. The most superficial cells lose their nuclei and cytoplasm, leaving masses of keratin.
Stratum cornuem (the keratin layer)	Composed of flat sheets of keratin. The protective surface coating of the skin.

Exfoliative cytology

Exfoliative cytology is widely used to collect material from the female genital tract, respiratory tract and urine bladder to check for infectious processes or premalignant or malignant changes in the respective organs (22). The method is also relevant within the field of dermatology. The epithelium constantly sheds cells because of cell renewal. Exfoliated cells can be collected for evaluation and provide information about pathological processes of the skin. Exudate and other types of secretion from epidermal processes can also be collected and may be useful to evaluate, such as bacteriological evaluation of ulcerated wet lesions.

Exfoliative cytology has been evaluated as a diagnostic tool in dermatology. In a diagnostic test meta-analysis *Bakis et.al* (13) reviewed exfoliative cytology as a diagnostic tool for BCC. They found that exfoliative cytology had a high diagnostic accuracy with a sensitivity and specificity of 97% and 86%, respectively. In addition, the use of cytology has an advantage in cases where punch biopsy may be considered inappropriate, as in cosmetically important sites as the face. In a prospective study done by *Vega-Memije et.al* (14) it was shown that exfoliative cytology has a lot of advantages as it is easy to perform, is non-invasive, time-efficient and reliable for differentiation of malignant skin tumours. However, disadvantages with the method are commented, the main one being that cytology does not give much information about tumour growth and subtypes. The authors concluded that cytology is very useful for rapid diagnosis of skin tumours, but in cases of aggressive BCC or suspicion of SCC, histopathological confirmation is advised before choosing treatment modality.

Skin-scrape cytology

Skin-scrape cytology, also known as abrasive cytology, is frequently used for direct sampling of cells from the cervix. Also, endoscopic instruments enable the possibility for direct cytological sampling from the respiratory and gastrointestinal tract (22).

This sampling technique is favourable in dermatology as it allows the opportunity to collect cells from a specific site. The skin-scrape technique allows contact with the deeper layers of the epidermis, thus cells others than those naturally shed can be collected. As early as in 1940 Tzanck used skin-scrape cytology for differential diagnosis of bullous skin disease (22). Following on from this, more studies on the use of skin-scrape cytology were carried out. In 1984 *Gordon et.al* (11) evaluated the use of cytology of cutaneous neoplasms. They obtained scrape smears from different neoplasms and compared these to histology from biopsy samples from the same lesions. With 89% compatibility, the authors concluded that cytodiagnosis of BCC is a reliable diagnostic method. In an other study, *Berner et.al* (7) investigated the accuracy of skin scrape cytology in the diagnosis of BCC and concluded that skin scrape cytology is reliable for the diagnosis of BCC. The method was put forward as of great advantage when non-invasive treatment modalities such as PDT are considered. Cytology, when used together with non-invasive therapeutic options, secures a cosmetically good outcome as is important in cosmetically challenging areas such as the face or in the treatment of young individuals. *Christensen et.al* (6) investigated the quality of skin-scrape cytology on lesions histologically verified as BCC and AK. They used both MGG (May-Günwald-Giemsa) and Pap (Papanicolaou) stains. It was concluded that cytological diagnosis for BCC and AK is reliable and both sensitivity and specificity was high. They also studied results of imprint cytology of BCC and AK, but in this study imprint cytology had a too low sensitivity to be considered as a reliable diagnostic tool.

Aspiration cytology

Aspiration cytology can be used on a great variety of sites and organs and can be applied to both palpable and non-palpable lesions. Examples are cysts of the neck, regional lymph nodes for metastatic evaluation and palpable lesions of the breasts. Aspiration cytology is therefore used within a great number of specialities (22).

In 1999 *Fang et.al* (18) described their findings with the use of fine needle aspiration cytology (FNAC) on BCC of the skin. They stated that FNAC is a good initial diagnostic procedure for BCC and that the method is best applied for nodular and ulcerative tumours. A study done by *Kate et.al* (17) also concluded that cytological examination of BCC with the use of FNAC is highly accurate and is a good method for initial evaluation of BCC.

Imprint cytology

Imprint cytology is mainly used in intraoperative procedures as it allows ongoing evaluation of the surgical field. This ensures a rapid and exact diagnosis in the surgical process. The method has been used in neuropathology for many years (22).

Regarding dermatological cases intraoperative cytology itself is not very relevant. However, the sampling technique of surgical tissue collection with the use of touch imprint, has been investigated within dermatological diagnostics as shown by *Christensen et.al* (6) in the section above. *Ramakrishnaiah et.al* (10) also studied the method of imprint cytology on ulcerated skin neoplasms by taking an imprint from the cut surface of a wedge biopsy specimen or from the resected margins of a surgical specimen. They found that the method was reliable for use on different skin malignancies such as SCC, however the method was

not useful for the diagnosis of BCC. Intraoperative imprint cytology can be used to evaluate resection margins to ensure adequate excision of skin tumours.

Liquid-based cytology

The ThinPrep® Pap test contains a liquid methanol-based solution for fixation. This allows immediate fixation of cells and the solution is thereby prepared and stained with Pap for visualisation of collected cells (23). In this study we used a Medscand®Cytobrush to sample cells for cytological evaluation. This is a soft brush normally used to sample cells from the endocervix. The broom-like construction allows collection of cells without tissue disfiguration (Figure 2).

The ThinPrep® Pap test is widely used in screening programs for cervical cancer and has shown good results with the tendency to produce higher sensitivity-rates than using the conventional Pap-smears (8). The use of LBC (ThinPrep® Pap test) in diagnostics of cervical cell dysplasia has shown several advantages. The method is more effective and easier to perform compared to conventional methods, and allows direct fixation of cells after collecting the sample. LBC with ThinPrep® Pap test has shown to produce a satisfactory number of representative cell samples. A great number of cells are transferred to the test container and thus eliminating the possibility of artefacts by air-drying which results in better distribution of the cells in the prepared sample (8, 9). The sensitivity of LBC tests compared to conventional Pap smears are in some studies controversial, however it has been shown that LBC decreases the frequency of inadequate smears (27).

Disadvantages in using LBC may be related to sampling errors such as unsatisfactory transfer of cells from the testbrush to the sampling container and blood cells contaminating the sample. Moreover, insufficient transfer of cells from the skin to the cytological brush is a potential issue. It is therefore advisable that a trained practitioner should perform the cytological sampling. A good test sample is important to achieve a desired high diagnostic accuracy (28).

Liquid-based cytology demonstrated in diagnostics of dermatological conditions

Morrison et.al (15) evaluated the use of LBC in combination with FNAC on lesions histologically verified as malignant melanoma (MM). The cytological findings of the LBC-smears were compared to the findings of conventional cytological smears stained with Pap, H&E (Hematoxylin&Eosin) or Romanowsky. The study showed that melanin was detected in higher proportions in liquid-based aspirates than conventional aspirates (15). In a study by *Onuma et.al* (20) using LBC in diagnosis of pemphigus vulgaris (bullous disease) of the vagina, advantages with use of LBC was demonstrated compared to histology and conventional smears. The initial evaluation, using a conventional Pap-test, was interpreted as SCC. Following this, a cervical biopsy and LBC-sample were taken from the same affected area. The histological evaluation of the tissue section confirmed the suspicion of pemphigus vulgaris. The cytological evaluation of the LBC-smear agreed with the histology diagnosis. Advantages of LCB over conventional Pap were better evaluation of chromatin patterns and of the nuclear membrane. Also, there were fewer red blood

cells, cell debris and mucous present, and malignant cells were more easily distinguished from benign cells using LBC (20).

Figure 2. The ThinPrep® Pap test and Medscand®Cytobrush (private photo).

Non-melanoma skin cancer

Basal cell carcinoma (BCC) and actinic keratosis (AK) are both classified as non-melanoma skin cancer (NMSC) where BCC accounts for 80% of this type of cancer (29). Following these, squamous cell carcinoma(SCC) is the second most common subtype of NMSC (30). Ultraviolet radiation (UV-R) is the single most important factor in the pathogenesis of developing BCC and AK. Intermittent exposure and exposure during childhood is important in developing BCC, whilst chronic exposure to UV-R is mainly linked to the development of AK (31, 32).

Both BCC and AK are frequently seen in fair-skinned individuals and the worldwide incidence is still rising (30). NMSC normally appears on sun-exposed skin such as the head and neck. Both locations are considered cosmetically important (33). NMSC is often not routinely reported to cancer registries, therefore accurate estimates of the incidence remains unclear. However, increased awareness of NMSC in the population due to focused intervention, information and screening most likely contribute to the rapid increase in reported cases of NMSC. During recent years, a greater number of patients have visited health-clinics with lesions suspected of skin cancer. NMSC is therefore a growing economic health burden in society, Australia, the US and the UK, in particular, are all countries with good documentation of the rapid increase in incidence and the growing health challenge this presents (34, 35). In the US AKs are second to acne as the most common reason for patients to consult a dermatologist (36). In Sweden more than 30 000 new cases of BCC are seen per year and in Norway close to a 1000 new cases of SCC are diagnosed each year (35, 37).

The incidence of skin cancer is more common in transplant recipients as compared to the general population. The frequency of AK is increased by 40% after 5 years of immunosuppression in comparison to the population in general. Some of these cases may progress to SCC. In the general population BCC is

more frequent than SCC. The ratio is reversed in transplant recipients using immunosuppressants with a ratio of SCC to BCC greater than one. Multiple carcinomas occur in more than 50% of transplanted patients with skin cancers (38).

Basal cell carcinoma

BCC is the most common cancer in Caucasians. Two out of three cases arise in the head and neck region, whilst remaining cases are seen predominantly on the trunk and extremities. Fair-skinned individuals are at greater risk of developing BCC (39). Other risk factors of importance are immunosuppression, exposure to arsenic, scars and hereditary disorders such as nevoid basal cell carcinoma syndrome and xeroderma pigmentosum. The average age at first diagnosis is 60 years, but BCC can also be found in younger patients (29). Clinically BCC is usually classified into three main subgroups: nodular BCC (nBCC), superficial BCC (sBCC) and morfea type BCC (mBCC) (40). Different classification systems exist where a variety of groups and subgroups of BCC are used. Common characteristics of BCC are related to changes in the basal cells of the epidermis showing sheets of uniform cells with enlarged spherical nuclei and scanty cytoplasm. Mitotic cells are scarcely observed (22, 40, 41). The patched/hedgehog intracellular signalling pathway plays a central role in both sporadic BCCs and nevoid basal cell carcinoma syndrome. This pathway is vital for the regulation of cell growth, and differentiation and loss of inhibition of this pathway is associated with development of BCC (42). Mutations in the vital tumour suppressor genes PTCH and p53 are characteristic in BCC. PTCH controls the proliferation and differentiation of cells as well as playing an important role in interaction between cells. An important regulator of the cell cycle is p53 and mutation of the gene inhibits initiation of apoptosis of UV-R damaged keratinocytes. BCC is known to be local invasive and slow growing, but hardly ever metastasises (30, 33). Metastasis has an estimated incidence of approximately 0.0028 – 0.55%. Its local invasive character can however reach underlying structures such as cartilage, nerves and vessels and cause significant damage (29). Suspicion of BCC should be raised when typical skin manifestations such as pearly pink or flesh coloured papules with telangiectasia and translucent or slightly erythematous lesions with rolled borders appear. BCC is occasionally accompanied by bleeding, scaling or crusting (33).

Actinic keratosis

AK is a common cutaneous skin lesion frequently appearing in fair skinned and blue eyed individuals and the prevalence increases with age (31, 39). Those with skin phototypes Fitzpatrick I and II are at greater risk of developing AK (43) (Table 4, 44, 45). Other important risk factors are frequent exposure to sunlight and occupations with a considerable amount of work outdoors (43). Treatment with radiation therapy is also a relevant risk factor (46). Protective factors are use of cosmetics and sunscreen, protective clothing and staying indoors during midday hours of strong sun exposure (43). Histologically AK is acknowledged as an epidermal neoplasm consisting of atypical epidermal keratinocytes (39). UV-R leads to damage of the cell DNA. The incidence of p53 mutations in white patients affected by AK has been found to be approximately 75-80% (46). The p53 gene is a tumour suppressor gene that encodes for a transcription factor. Its normal function is to regulate the cell cycle, repair damaged DNA and to induce apoptosis. UV-induced mutations of p53 results in loss of function with survival and further division of damaged cells.

This leads to accumulation of additional mutations (46). However progression to SCC is extremely rare and although exact calculations are difficult to do due to insufficient data, approximately 1-10% of AKs progress to SCC (46). Clinical manifestations of AK are known as scaly elements with erythematous papules or plaques (31). The lesion is often easily suspected by palpation and gently stroking a finger over the affected area. A rough, scaly, sandpaper-like surface is typically recognised. An interesting discovery is the relation between AK and transplant patients. In general 40% of transplant patients develop premalignant or malignant skin tumours within 5 years of receiving the transplant (43).

Table 4. Fitzpatrick's skin phototypes.

Skin type	**Unexposed skin colour**	**Phenotypic characteristics**	**Reaction to sunlight**
I	White	Freckles common. Usually blue eyes and blond hair	Always burns, never tans
II	White	Usually blue eyes and blond hair	Always burns, minimal tan
III	White to olive	Eye and hair colour vary	Burns minimally, gradually tans
IV	Light brown	Usually dark hair and brown eyes	Burns minimally, tans well
V	Brown	Usually dark hair and brown eyes	Very rarely burns, tans profusely
VI	Dark brown to black	Dark hair and eyes	Never burns, tans deeply

Diagnostics of Basal cell carcinoma and Actinic keratosis

Gold standard

The European Dermatology Forum Guidelines for intervention of AK (47) and BCC (48) have a detailed section on diagnostic recommendations for AK and BCC and serve as guidelines for practitioners.

Basal cell carcinoma

Clinical evaluation is very useful and based on typical clinical appearance of the lesion, a BCC diagnosis is suspected clinically but is usually confirmed by histology. Histological evaluation is useful as it provides information on subtypes of BCC. Dermatoscopy may be used for clinical evaluation by dermatologists. Experience and training in use of the tool is desired as one must know the clinical characteristics of BCC to be able to set an accurate diagnosis(Figure 3, 49). Confocal microscopy is mentioned as an emerging non-invasive tool in dermatology and offers in-vivo diagnosis of skin tumours at near histological resolution. It has shown high diagnostic accuracy for the diagnosis of BCC (48) (For details on dermatoscopy and confocal microscopy, see section *Other non-invasive diagnostic modalities* below) .

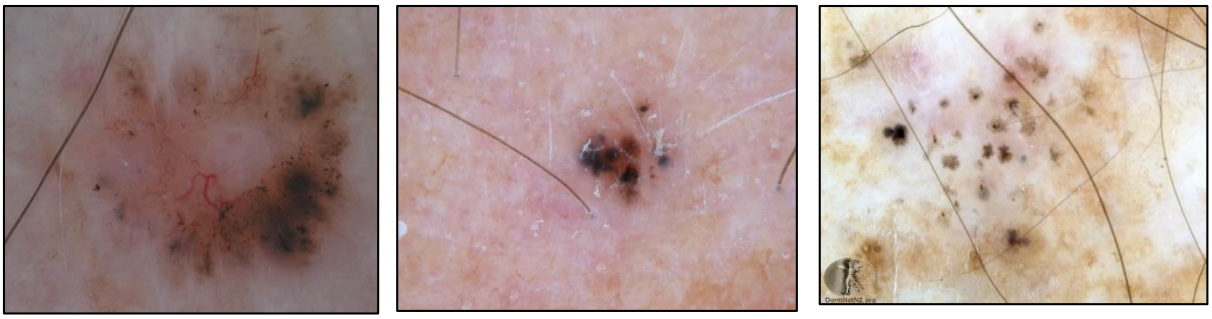


Figure 3. Dermatoscopic features of BCC. From left: first picture shows aborizing telangiectasia, flecks of pigment and blue-grey globules. Second picture shows blue ovoid nests. Third picture shows spoke-wheel areas.

Actinic keratosis

Diagnosis is usually based on clinical examination of the skin with typical characteristics of AK present such as scaly elements with erythematous papules or plaques. Patient history of typical risk factors is also important. The concurrent use of dermatoscopy and confocal microscopy may help raise the clinical suspicion of AK. Typical dermatoscopic features of AK are shown in Figure 4 (50). Biopsy of AK is recommended e.g. in cases of unclear clinical diagnosis, suspicion of progression to malignant disease or unresponsive AK lesions (47).

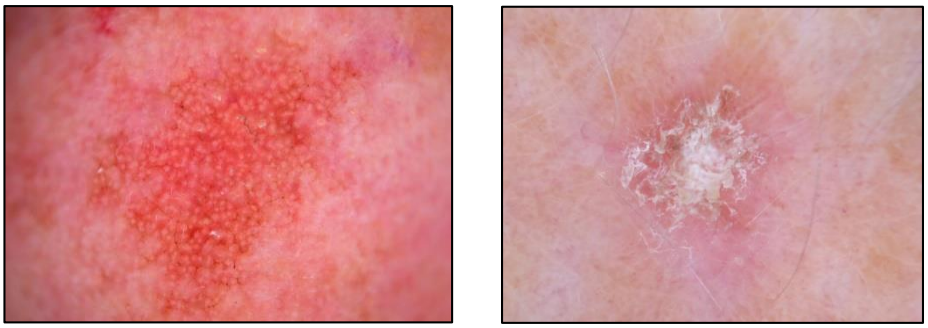


Figure 4. Dermatoscopic features of AK. From left: first picture shows pigmented lesion with strawberry pattern (erythematous pseudonetwork with yellow hair follicles) and is diagnostic for AK. Second picture shows non-pigmented AK.

Other non-invasive diagnostic modalities

There are several other diagnostic modalities considered useful in the evaluation of NMSC. The main disadvantages with these are the lack of availability or the need for practice and maintenance of skills to secure good quality of use.

Confocal microscopy

Confocal microscopy provides information on dermal morphology and allows visualisation of cells and tissue in slices. A two-dimensional picture is formed with the help of light providing high resolution images (51). A disadvantage with the method is the poor ability to distinguish between different types of skin cancer (52).

Dermatoscopy

Dermatoscopy involves the use of a handheld microscope (dermatoscope) consisting of a light source and magnifying glass. Different features of the lesion not visible to the human eye are evaluated. It is considered a good tool in clinical practice as it can be used to visualize pigmentation of the lesion and also determine if the lesion is malignant or benign (51, 53). Dermatoscopy is useful in aiding the clinical diagnosis and different criteria have been developed to raise the suspicion of AK and BCC (54, 55). It may be applied to better differentiate benign from malignant skin lesions and to detect tumours at an early stage (55). If clinical or dermatoscopic features are inconclusive or uncertain, biopsy and histopathological evaluation should be performed (54, 55).

Ultrasonography

Ultrasonography is based on the fact that different tissues absorb and reflect soundwaves in different manners and a two-dimensional picture is formed. The modality can be used to determine the thickness, borders and texture of subcutaneous masses. Regional lymph nodes are investigated by ultrasonography to evaluate and monitor the likelihood of metastasis (51). High frequency ultrasonography (20 mHz) is considered a good tool when evaluating the thickness of a suspected lesion (56).

The benefits of cytology compared to other non-invasive diagnostic modalities

All modalities above are non-invasive, and when used together with cytology they represent methods for non-invasive evaluation of non-melanoma skin cancer suspected lesions. The advantage of cytology is that it is readily accessible in clinical practice, easy and rapid to perform and prepare, and may provide cell material from the whole lesion surface for microscopic evaluation.

The advantages of cytology in diagnosis of BCC and AK

Evaluation by histopathology of skin cancer suspected lesions is today considered the gold standard in diagnostics. Tissue samples are usually collected with the use of biopsy (57). The method requires use of local anaesthetics and may leave scarring on the site of biopsy. However, non-invasive methods such as cytology, are desirable due to the establishment of new non-invasive treatment options of non-melanoma skin cancer such as photodynamic therapy (PDT) and imiquimod (31, 58). PDT is based on the use of a photosensitizer (aminolevulinic acid (ALA)/methyl aminolevulinate (MAL)) and red light activation (632 nm) to induce damage of the tumour cells with limited formation of post-treatment scarring (58). Imiquimod binds to Toll-receptor 7 and 8 acting as a topical immunomodulator and thereby inducing damage of tumour cells, without post-treatment scarring (59). Several factors may influence the decision to pursue nonsurgical alternatives in treatment, including lower overall costs and a more acceptable cosmetic outcome (58). It is however important to remember that knowledge of recurrence rates and type of lesion should be carefully considered when choosing treatment modality (60). Surgical procedures in treatment are highly effective, but can be painful postoperatively, result in loss of healthy tissue and cause scarring. As a great number of lesions develop on cosmetically important sites, such as the face and neck, it is important to consider the impact of surgical treatment on patients. The surgical procedure itself may lead to psychological discomfort as well as reduced quality of life due to scarring (61). As cytology is a minimal-invasive procedure, this method may be beneficial in diagnostics where topical treatment options are

preferred, resulting in an overall positive cosmetic outcome in both diagnostics and treatment. The usefulness of cytology in diagnostics following treatment with PDT was investigated by *Berner et.al* (7). Their study demonstrated that PDT is a simple and cost-effective treatment for nBCC and sBCC and provides a good cosmetic outcome. The use of skin-scrape cytology is reliable in diagnostics of BCC and has the advantages of being fast and inexpensive (7).

Common treatment of Basal cell carcinoma and Actinic keratosis

Basal cell carcinoma

The following treatment modalities and recommendations are based on the European Dermatology Forum Guidelines (48). An overview of the therapeutic options is presented in Table 5.

Surgical Excision

Surgical excision involves surgical removal of the lesion and surrounding tissue with a scalpel. Excision is the standard treatment of BCC and should always be used on high-risk tumours, such as aggressive subtypes and infiltrative growth (mBCC). The width of surgical margins varies and relies on tumour characteristics, size and site. With satisfactory surgical margins, the tumour is successfully eradicated in more than 95% of cases (48). Recurrence rates vary from 2-8% at 5 years after surgery. Tumours that are not sufficiently removed, especially high-risk BCC and lesions incompletely excised at deep margins, should be re-excised (48).

Mohs micrographic surgery

Mohs micrographic surgery is a surgical technique with systematic histological evaluation of excision borders using intraoperative frozen section. The method is of great advantage in use as it reduces tissue disfiguration of normal skin (29, 48). Treatment with Mohs yields extremely high cure rates and the advantage, compared to surgical excision, is prevention of uninvolved tissue. The method is recommended for use on high risk and recurrent BCC. Overall cure rates of BCC with Mohs range from 97-99% for primary tumours to 93-98% for recurrent tumours after 3 to 5 years of follow up (48). A disadvantage with the method is its cost- and time-consuming nature due to ongoing histological evaluation of intraoperative frozen sections during surgery. A pathologist will also need to be present during the procedure (48).

Electrodissection and curettage

The technique involves curettage (skin-scrape technique) of the tumour before dissecation (coagulation) using an electrode in direct contact with the tissue. ED & C can be used on friable tumours such as nBCC and sBCC. The method is not recommended for use on aggressive subtypes of BCC such as mBCC or in cases of recurrent BCC. Recurrence rates after 5 years vary, but are estimated to be 3.3% in low risk BCC and 18.8% in high risk BCC (48).

Cryosurgery

Cryotherapy is recommended for use on low risk BCC and involves the use of liquid nitrogen sprayed directly onto the lesions. It induces selective necrosis and uses the effect of extreme cold to cause tissue damage. Prior to cryotherapy the lesion can be curetted carefully to reduce its mass. There is no single

standard technique or protocol for use, but double freeze/thaw cycles are generally recommended for treatment of BCC. Recurrence rates are highly variable and range between 8-40%(48).

Laser

Carbon dioxide (CO_2) laser is a less frequently used method for treatment of BCC. This procedure provides a bloodless field, minimal postoperative pain and good postoperative appearance. Using CO_2 laser may be effective in treatment of low risk BCC, especially in patients with bleeding conditions. The main disadvantage with the method is availability and great variance in reported recurrence rates (48).

Radiotherapy

Radiotherapy is an efficient method of treatment for BCC and an option when location does not allow satisfactory surgical excision, when the patient is not eligible for surgery or declines surgery, or when treating a recurrence of earlier excised BCC (29, 48). It does however require histological confirmation of diagnosis prior to treatment. Radiotherapy is also useful in the treatment of incomplete excised lesions, nBCC of the head and neck under 2 mm and BCC with invasion of bone and cartilage. Radiotherapy is contraindicated in genetic syndromes predisposing to cancer such as nevoid basal cell syndrome and xeroderma pigmentosum, in first-line treatment where excision is possible, in patients aged under 60 years and for treatment of mBCC. Treatment of sites such as ears, hands, feet, legs and genitalia are also contraindicated. Cure rates vary somewhat, but careful patient selection can result in very high cure rates with estimated numbers ranging between 90-96%. The estimated recurrence rate for all patients with BCC is approximately 15,8% (48).

Photodynamic therapy

PDT is licensed for treatment of BCC in many European countries and involves the use of a photosensitive cream (ALA/MAL) followed by red light activation. The method is appropriate for treatment of sBCC and small, primary nBCC and has the advantage of superior cosmetic outcome. Moreover, PDT is an option for treatment in immunosuppressed patients and transplant recipients. Anaesthesia and use of pain relief is used to a variable extent, but is necessary in many cases due to the pain/burning sensation often experienced during light treatment. Local reactions at the site of treatment often occur. These are reported as erythema, oedema, erosions and crusting. Two cycles of PDT is recommended for treatment of BCC. Short term clearance rates are between 92-97% and recurrence rates approximately 20% 5 years after treatment (48).

Imiquimod

Imiquimod is a topical cream for treatment of low risk superficial BCC. The recommended treatment period is 6 weeks with cream application 5 times per week. The main advantage is a non-invasive treatment of tumours with prevention of the surrounding tissue, thus yielding a favourable treatment outcome. Home-based treatment is possible. Transient local site reactions with irritation of surrounding skin is common with ulcerations, erosions and sensation of pain. Clearance rates depend on duration of treatment period and vary between 42-81% (48).

New treatments for advanced BCC

Specialized drugs have been developed for management of uncontrolled/advanced local disease and for patients with metastatic BCC (48). Vismodegib, a new oral hedgehog pathway inhibitor, has proven to be

effective treatment for advanced BCC. However, patients experience several side-effects when undergoing treatment (62).

Therapeutic tradition of BCC in Scandinavia

Sweden is the only Scandinavian country with prepared national guidelines. Recommendations here are quite similar to the European Dermatology Forum Guidelines, but there are some variances due to differences in local therapeutic traditions and professional experience. In short, these are the recommendations of the Swedish Guidelines (63):

- Surgical excision, including Mohs micrographic surgery, is the first-line treatment for all facial BCCs, but curettage and cryosurgery may be used as an alternative first-line therapy of nodular BCC localized to nose, ears, and eyelids.
- PDT and topical imiquimod can be used in facial superficial BCC as a second-line modality.
- For tumours localized outside the head and neck area, several treatment choices are recommended based on the aggressiveness, size, and localization of the tumour.
- Non-invasive treatments are superior in terms of cosmetic outcome and are more often used in the situation of multiple superficial BCC.

Helsing et.al (63) investigated the treatment choices of Scandinavian dermatologists with regard to BCC and found that treatment of choice differed substantially between dermatologists from the three Scandinavian countries Sweden, Norway and Denmark. Options for treatment in each individual case were surgical excision, topical immunotherapy, PDT, cryotherapy, ED & C, curettage and radiotherapy. Swedish dermatologist more often chose excisional surgery compared to Norwegian and Danish dermatologists. Norwegian dermatologists more often chose PDT compared to Swedish and Danish dermatologists. Very few Swedish dermatologists chose radiotherapy as a treatment option (63).

Topical 5-FU is a treatment method that is commented in the European Dermatology Forum Guidelines, but is not approved for treatment in Norway. It has earlier been evaluated for use on both BCC and AK (47, 48). Ingenol mebutate and topical retinoids are also treatment options for BCC available, however there are no present recommendation for the use of these(48). Mohs micrographic surgery is limited in many countries and has just recently been introduced in Norway (63).

Table 5. Overview of interventions for BCC.

Treatment modality	Type of BCC	Comments
Surgical excision	High risk and aggressive types of BCC. Primary BCC. Recurrence of earlier excised BCC.	Gold standard. High clearance rates. Scarring.
Mohs micrographic surgery	High risk BCC. Recurrence of high risk BCC and aggressive subtypes of BCC.	Prevention of uninvolved tissue. Extremely high clearance rates. Not a standard routine at many hospitals due to advanced technique. Time-consuming. Scarring.
ED & C	Low risk BCC. nBCC, sBCC.	Scarring.
Cryosurgery	Low risk BCC.	Easy to perform, cheap and highly available method. Scarring and hypopigmentation on site of treatment.
Laser	Low risk BCC.	Non-invasive. No bleeding – an advantage in patients with bleeding/coagulation disorders. Poorly reported recurrence rates.
Radiotherapy(RT)	Certain primary BCC where surgery is not possible. Recurrent BCC with the exception of recurrence after previous RT. Incomplete excision of BCC. BCC with invasion of bone and cartilage.	Before choosing RT, thorough assessment of patient history, evaluation of contraindications and other options for treatment must be taken into consideration. Radiation has carcinogenic properties.
Imiquimod(Aldara®)	Low risk BCC. Small sBCC and nBCC.	Non-invasive. Good cosmetic outcome. Home-based treatment possible. Local reaction on site of treatment and surrounding tissue. Patient compliance important.
Photodynamic therapy	Low risk BCC. nBCC and sBCC.	Non-invasive. Superior cosmetic outcome. Local reaction and pain on site of treatment.
Ingenol mebutate(Picato®)	No present recommendation available.	Non-invasive. Home-based treatment possible. Local reaction on site of lesion and surrounding tissue.
Topical retinoids(Tazaratone®)	No present recommendation available.	Non-invasive. Home-based treatment possible. Ongoing trials to evaluate its effect, little information about the treatment available.
5-FU	Has earlier been evaluated for treatment of BCC.	No longer approved for use in Norway. Available for use in other European countries.
Antineoplastic drugs Vismodegib(Erivedge®)	Therapeutic option for locally advanced and metastatic BCC.	Often old patients with comorbidity. Side effects. New treatment.

Actinic keratosis

Several options are available for treatment of AK. Choice of modality depends on type and number of lesions. The following recommendations are based on the European Dermatology Forum Guidelines(47). Strength of recommendation for each single method is based on assessment of outcome-rates and clearance-rates reported in published studies (Table 6, 47). An overview of the treatment modalities is listed in Table 7.

Surgical excision

Surgical excision is not routinely used for management of AK (47).

Curettage

Curettage, using a skin-scrape device, is particularly useful to treat single hyperkeratotic lesions of the extremities. It can also be used for treatment on immunosuppressed patients. Despite long experience with this modality, reliable information to document its effect is scarce. However, curettage is understood as a useful tool to reduce the thickness of hyperkeratotic lesions before use of other treatment modalities to secure satisfactory penetration of the treating agent. Curettage is also used for field preparation before treatment with PDT (47).

Cryotherapy

Cryotherapy is a widely used and well-established method for treatment of AK. It is often used in an office-based setting, as it is easy to use, cheap and quick to perform. The thickness of the lesion is however an important factor to as how well the lesion responds to therapy. Recommendations for use of cryotherapy are mainly for patients with single AK lesion. Compared to imiquimod and PDT, cryotherapy is often inferior with respect to achievement of a favourable cosmetic outcome. The use of cryotherapy may cause local reactions to the treated area such as erythema, oedema and blistering. The occurrence of prolonged wound healing, scarring and hypopigmentation may be expected post treatment (47).

Laser

The use of carbon dioxide (CO^2) laser is an efficient method with respect to long-term efficacy. Effect and safety of use is dependent on user experience. Disadvantages with the method are risk of infections, scarring and hypo/hyper-pigmentation post-treatment. Immunocompromised patients are more susceptible to infections and the use of CO_2 laser is therefore not recommended. Overall, laser is not a highly recommended method for treatment of AK. The same recommendations apply for the use of Er:YAG-laser which uses erubium as lasing media. The difference between the two is that Er:YAG-laser does not penetrate the epidermis as well and should therefore not be used on hyperkeratotic lesions (47).

Topical diclofenac

The use of this treatment modality is limited and topical diclofenac has much poorer long-term efficacy compared to other topical treatments. The treatment duration is long (60-90 days) with application twice a day, which imposes a challenge to patient compliance and practicability of the method. Topical diclofenac may be used in treatment of multiple AK (47).

Imiquimod

Imiquimod is a topical cream for direct application of lesions. Different concentrations of the cream exist (2,5%, 3,75% and 5%), representing use on a variation of AK lesions with different strength of recommendation. The use of imiquimod 2.5% has the poorest outcome in studies. Imiquimod can be used for treatment of all types of AK lesions, see Table 6 for recommendations. Advantages with the method is its non-invasive character and good cosmetic outcome. Local reaction on the site of treatment will most likely occur, such as erythema and ulcerations. Recommended treatment period is a duration of 4 weeks with application 3 times per week (5% imiquimod) (47).

Ingenol mebutate

Ingenol mebutate is a recently available treatment option for topical therapy of AK. It can be used for treatment of single AK lesions, but the highest recommendation is treatment of multiple AK lesions. No recommendation can be made for immunosuppressed patients due to the lack of experience from this patient group. The biggest advantage compared to other topical alternatives is the short duration of treatment period with application once a day for 2-3 days (47).

Photodynamic therapy

PDT involves the use of a photosensitive agent in combination with red light activation. Two types of PDT are used in dermatological practice; ALA-PDT and MAL-PDT, the difference being the type of photosensitive agent used. ALA-PDT and MAL-PDT is highly recommended for use on multiple AK lesions and field cancerization. Lamps are placed in different angles and directions to cover the desired area for treatment. Both types of PDT is used for treatment of all types of AK lesions in general, but the use of MAL-PDT is recommended for use in immunosuppressed patients due to better reports on efficacy and tolerability (47). One cycle of treatment is recommended when treating AK. The greatest advantage with PDT is the superior cosmetic outcome. Pain/burning sensation may be felt during treatment. This pain is often greater than the pain experienced with treatment of BCC. Also, local reaction on and around the treated area is expected post-treatment (47).

Combination of interventions

Combination of intervention methods is also an option and is individually evaluated for each patient. Choice of treatment and combinations of treatments may also be subject to differences in therapeutic traditions between different dermatologists.

Table 6. Strength of recommendation in interventions of AK. ↑ ↑ strong recommendation for use, ↑ weak recommendation for use, 0 cannot make a recommendation, ↓ weak recommendation against use.

		single AK lesions ≥ 1 and ≤ 5 palpable or visible AK lesions per field or affected body region	**multiple AK lesions** ≥ 6 distinguishable AK lesions in one body region or field	**field cancerization** ≥ 6 AK lesions in one body region or field, and contiguous areas of chronic actinic sun damage and hyperkeratosis	**Immunocompromised patients with AK** AK at any of the mentioned severity degrees and a concomitant condition of immunosuppression
		Sun protection in all patient subgroups!			
Strength of recommendation	↑↑	Cryotherapy	0.5% 5-FU 3.75% imiquimod Ingenol mebutate 0.015% / 0.05% MAL-PDT, ALA-PDT		-
	↑	Curettage* 0.5% 5-FU, 5% 5-FU 0.5% 5-FU + 10% SA* 3.75% imiquimod 5% imiquimod ingenol mebutate 0.015/0.05% ALA-PDT, MAL-PDT	Cryotherapy** 3% diclofenac in 2.5% HA 5% 5-FU 0.5% 5-FU + 10% SA* 5% imiquimod, 2.5% imiquimod CO2-laser, Er:YAG-laser		cryotherapy** curettage* 5% 5-FU 5% imiquimod*** ALA-PDT, MAL-PDT
	0	3% diclofenac in 2.5% HA 2.5% imiquimod CO2-laser, Er:YAG-laser	Curettage*		3% diclofenac in 2.5 % HA 0.5% 5-FU 0.5% 5-FU + 10% SA 2.5% imiquimod, 3.75% imiquimod Ingenol mebutate 0.015%/0.05%
	↓	-	-		CO2-laser, Er:YAG-laser

* discrete, hyperkeratotic AK lesions
** single or multiple discrete AK lesions, not for treatment of field cancerization
*** For immunosuppression, different clinical situations may exist, e.g. iatrogenic medical immunosuppression after organ transplantation, iatrogenic medical immunosuppression because of autoimmune disorders, immunosuppression due to other reasons (hematologic disorders, AIDS etc). Depending on the underlying disease, special care has to be given to the selection of the treatment to avoid (auto-) immunstimulation that may lead to a worsening of the underlying condition.

Table borrowed from the European Dermatology Forum Guidelines for treatment of AK (47).

Table 7. Types of intervention for AK.

Treatment modality	Type of lesion	Comments
Curettage	Hyperkeratotic AK. Single AK lesion. Immunosuppressed patients.	Useful in combination with other treatment modalities to ensure satisfactory penetration of treating agent on hyperkeratotic lesions. Scarring.
Cryotherapy	Method of choice for treatment of single AK lesions. Can be used on multiple AK lesions and in immunosuppressed patients.	Highly available, quick and easy to perform, cheap. Scarring and hypopigmentation on site of treatment
Laser(CO2 and Er:JAG)	Multiple AK lesions.	Not frequently used. Risk of infection, scarring and hypo-/hyperpigmentation following treatment.
5-FU(topical)	Can be used on all AK lesions.	Not approved for use in Norway. Available in other European countries.
Topical diclofenac(Solaraze®)	Multiple AK lesions.	Non-invasive. Home-based treatment possible. Long duration of treatment period. Patient compliance important.
Imiquimod(Aldara®)	All types of AK lesions.	Non-invasive. Home-based treatment possible. Good cosmetic outcome. Local reaction on site of treatment and surrounding tissue. Patient compliance important.
Ingenol mebutate(Picato®)	Multiple AK lesions. Single AK lesions. Not recommended for treatment in immunosuppressed patients.	Non-invasive. Home-based treatment possible. Fairly new treatment method. Local reaction on site of treatment and surrounding tissue. Patient compliance important.
Photodynamic therapy	All types of AK lesions.	Non-invasive. Superior cosmetic outcome. Local reaction on site of treatment and surrounding tissue.

MATERIALS AND METHODS

This was a prospective, blinded, single-centre pilot-study and the study population consisted of patients referred to the outpatient clinic for PDT at the Department of Dermatology, St. Olavs Hospital, Trondheim University Hospital. All lesions were verified as AK and BCC by histology and were either primary AK or BCC. Exclusion criteria were pregnancy, breast-feeding, immunocompromised, transplant recipients and children under the age of 18. Morfea type BCC were also excluded from the study.

Inclusion of patients

Patients referred to PDT-treatment are treated every Monday at the Department of Dermatology, St. Olavs Hospital, Trondheim University Hospital. The majority of these patients have AK and BCC diagnoses which should be verified by histology prior to referral. Study investigators (SI, EC) attended 15 Mondays to enrol patients into the study (Table 8). Many of the patients did not meet the study criteria for the following reasons: no histology available, recurrent lesion, immunosuppressed patient or second time treatment with PDT. The number of days and hours spent at the outpatient clinic did not yield the number of samples expected prior to initiation of the study. Cancelled PDT-treatment due to illness and public holiday, as well as relevant patients not showing up to their appointment, also eliminated eligible patients.

Table 8. Study investigator attendance at PDT-treatment days and number of cytological samples included per day.

Date of attendance	**Number of samples included**
September 7th 2015	1
September 21st 2015	1
September 28th 2015	1
October 5th 2015	0
October 12th 2015	0
October 19th 2015	1
October 26th 2015	1
November 2nd 2015	1
November 9th 2015	0
November 16th 2015	0
November 23rd 2015	1
November 30th 2015	1
December 7th 2015	3
December 14th 2015	1
December 21st 2015	1
Total days: 15	**Total samples: 13**

Sampling and preparation

Biopsy of the lesion was performed prior to inclusion. Before sampling, the patient received local anaesthesia on the site of the lesion before a curette was used to gently remove superficial keratinizing cells in preparation for PDT (Figure 5). A Medscand®Cytobrush was used to harvest cell material from the rest of the suspected lesion. The brush was placed on the skin surface, pressed gently downwards while rotated with a two-fold passage over the lesion surface (Figure 6). This process took less than one minute to perform. To ensure good sample quality, the brush was quickly transferred to the ThinPrep® container and rotated firmly approximately 10 times, both clockwise and anti-clockwise as well as vertically against the container wall. The sample was taken to Section of Cytology at the Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim University Hospital for further preparation and evaluation. Preparation was performed with the use of a liquid-based instrument, Thinprep® 2000 (Figure 7) (Appendix 2), and staining done in the staining machine, Gemini AS (Appendix 3). Preparation was completed using the machine Thermo Scientific Microm CTM6 (Appendix 4) for cover glass protection of the specimen (Table 9 for overview). An experienced pathologist and cytologist evaluated each sample individually using light microscopy (Figure 8). The researchers evaluating the cytological samples had no knowledge of AK and BCC diagnoses verified by histopathology.

The special preparation machine, Thinprep® 2000, uses polycarbonate filters (Figure 8). By rotation the filters homogenize the sample and ensures satisfactory distribution of the cells. The cells are collected on the surface of the filter, which is then pressed against a slide to transfer the cells onto it. The staining machine, Gemini AS, stains the samples automatically with the use of a modified Papanicolaou staining method (22).

Figure 5. Curettage after injection of local anaesteshia (Photo Eidi Christensen).

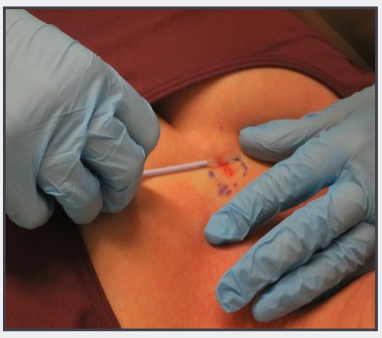


Figure 6. Cytological sampling with Rover®Cervexbrush (Photo Eidi Christensen).

Figure 7. The machine Thinprep® 2000 where the cytological smears were prepared (Photo Eidi Christensen).

Figure 8. The polycarbonate filter to which the cells attach. Cells are transferred from the filter to a glass slide by pressing the filter against the slide (Photo Eidi Christensen).

Table 9. The main steps in sample collection, preparation and staining.

Step	Procedure
1	A photo of the lesion was taken in relevant cases to include photo-material of AK and BCC lesions to the study.
2	Local anaesthesia (Xylocain-adrenalin® and/or Marcaine®) was injected into the treatment site.
3	Curettage and/or silver tape was used to remove crusts and cells from the stratum corneum. This in order to enhance the probability of collecting viable and representative tumour cells from the lesion.
4	The Medscand®Cytobrush was applied to the skin for cytological sampling.
5	The sample was delivered to the Department of Pathology and Medical Genetics for preparation and evaluation.
6	The sample was prepared in the preparation machine Thinprep®2000.
7	A modified Papanicoulaou staining method using the staining machine Gemini AS, coloured the samples automatically.
8	The sample preparation was completed using the cover glass machine Thermo Scientific Microm CTM6 .

Three of the samples were sparse in epithelial cells and rich in erythrocytes, and the diagnostic value was therefore not satisfactory. In such cases the investigators (CV, ME) employed a preparatory technique used on bloody LBC samples from the cervix for lysis of erythrocytes. The relevant cell samples were first added 10% acetic acid and then prepared once again using the same method as described earlier.

Cytological evaluation

Two investigators evaluated the cell samples (CV, ME) without knowledge of clinical features or histopathological diagnosis (Figure 8). If disagreement upon diagnosis occurred, this was registered and the investigators collaborated on deciding a final diagnosis. The quality of each cell sample was evaluated according to pre-set criteria (Appendix 5). Amount of cells present on the slide and quality of the prepared cells were observed to decide whether the sample was satisfactory or not. Samples were classified as BCC, AK, other diagnosis or non-evaluable material. Smears showing a great number of erythrocytes were registered and new slides were prepared adding 10% acetic acid before final preparation. The samples were evaluated once again and a new diagnostic proposal made.

Cytological diagnosis of AK

There is limited experience of cytological examination of AK and the main problem seems to be difficulty in obtaining representative cells by cytological sampling. Cells from the deeper layers are challenging to harvest and as a result, the specimens mainly consist of keratinized cells with few cells from the deeper layers. This highlights the importance of good sampling technique. The characteristics of AK are described as single cells and groups of dyskaryotic keratinocytes with uneven contours (25). A high N/C-ratio (nucleus/cytoplasma-ratio) is present and the cells have coarse chromatin and occasional prominent nucleoli. Cells in the deeper layers of the epidermis have basophilic cytoplasm while superficial and more differentiated cells have more eosinophilic cytoplasm due to keratinizing cells. This is used in the diagnosis of AK. Compared to cells in BCC, the cells are more differentiated as the changes are present in the more superficial layers of the epidermis (22).

Cytological diagnosis of BCC

Cytological characteristics involve cohesive, sometimes anastomosing, usually flat sheets or clusters of various sizes, composed of small epithelial cells with scanty cytoplasm. They have high N/C- ratio, basophilic cytoplasm and peripheral cells often form a palisade. The nuclei are round to oval with a smooth border, hyperchromatic with fine granular chromatin pattern, and one or two small distinct nucleoli. The single cells show similar cytological features, occasionally the nuclei are flattened, indented or angulated with coarser chromatin pattern. Additional features seen in some cases include the presence of mucinous material, squamous and sometimes keratinized tumour cells. The cells are small and poorly differentiated as they are basaloid cells (22).

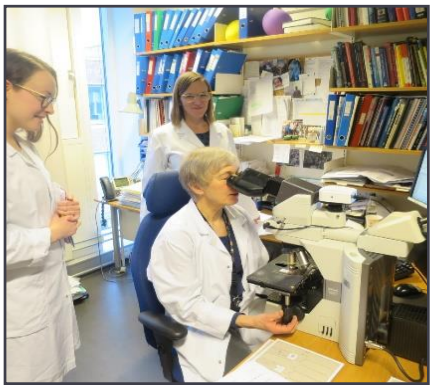


Figure 8. Evaluation of the cytological slides using light microscopy (Photo Eidi Christensen).

Histology: the gold standard

The diagnosis in the histopathology report served as the diagnostic gold standard. Histological features of AK involve changes in the epidermis that show either hypertrophy or atrophy with scattered nuclear abnormalities in the epithelial cells and peripheral palisading is often present (22). In BCC histologically the tumour is derived from the basal layers of the epidermis and is composed of solid, anastomosing sheets or strands of uniform small cells with spherical nuclei and scanty cytoplasm.

Diagnostic evaluation and statistical methods

The diagnostic accuracy of each cytological sample was evaluated by comparison to the histopathology report which was defined as the gold standard. Only one lesion per patient was included. The cytological results were grouped into four categories: BCC, AK, other diagnosis and non-evaluable material. The cytological test performance was expressed as point estimates for sensitivity and specificity with exact 95% confidence intervals. Sensitivity means the number of smears with positive cytological BCC or AK diagnoses among those histologically verified as BCC or AK, respectively. Specificity is defined as the number of smears with cytological diagnoses of not BCC or not AK among those with histological diagnoses of not BCC or not AK, respectively. A confidence interval is the range in which the true value of estimate lies within with a certainty of 95%.

Ethics and safety

The study was approved by the Regional Committee of Medical Research Ethics (REK) on May 20th 2015 (2015/621 REK) (Appendix 1). Patients were verbally and written informed about the study design and procedure for sample collection. Consent forms were handed out, read and signed by both patient and practitioner, each receiving one copy to keep (Appendix 6, Appendix 7). Study-information collected was stored in a locked room and only the project leader had direct access to the information. All tests and

photos included into the study were handled anonymously by labelling each test with the description CYT-BAK and its relevant number, ensuring that cytological tests could not be connected directly to a patient. Patients were insured through the Norwegian System of Patient Injury Compensation (NPE). The patient samples collected were taken under routine settings for PDT-preparation. The patients included into the study were handled in the same way as every other patient undergoing PDT. The treating doctor followed standard routines for PDT and the only intervention from the study investigators were when using the soft cytological brush on the relevant lesion. Little extra time was used, nor alterations made to the preparation-routines and no extra discomfort or adverse effects were inflicted on the patients.

RESULTS

Altogether 13 lesions (8 BCCs, 5 AKs) from 9 female and 4 male patients were included (Table 10). The majority of the lesions (n=8) were located on the head. The remainder were located on the upper thorax region (n=3) and on the extremities (n=2). Examples of corresponding clinical, histopathological and cytological images from one BCC and one AK are given in Figures 9 and 10.

The cytological and histopathological results agreed in 8 of the 13 (61.5 %) cases; in 6 of 8 BCCs and in 2 of 5 AKs (Table 11).

Two of the thirteen samples initially evaluated as non-diagnostic were added 10% acetic acid before they were found representative on second evaluation. Sensitivity and specificity for cytological diagnosis of BCC, was 75% and 100%, respectively. Sensitivity and specificity for cytological diagnosis of AK, was 40% and 100%, respectively. For more detailed information see tables 12 and 13.

Table 10. Baseline lesion characteristics and diagnosis of AK and BCC.

CYT-BAK Number	Gender	Location	Histological subgroup/grade of atypia	Histological diagnosis(gold standard)	Cytological diagnosis
1	♀	Left shoulder	nodular	BCC	BCC
2	♂	Left cheek	superficial	BCC	BCC
3	♀	Right posterior thorax	superficial	BCC	Non-evaluable
4	♂	Left anterior thorax	superficial	BCC	BCC
5	♀	Left chin	moderate atypia	AK	Non-evaluable
6	♀	Left chin	not specified	BCC	BCC
7	♀	Sternum	superficial	BCC	BCC
8	♀	Left upper lip	nodular	BCC	Non-evaluable
9	♀	Left cheek	moderate atypia	AK	Non-evaluable
10	♂	Right anterior calf	severe atypia	AK	AK
11	♀	Left cheek	severe atypia	AK	AK
12	♂	Temple, lateral to the left eye	nodular	BCC	BCC
13	♀	Inferiolateral to the right eye	moderate atypia	AK	Non-evaluable

Table 11. Diagnosis by LBC (ThinPrep® Pap test) and histology for AK and BCC.

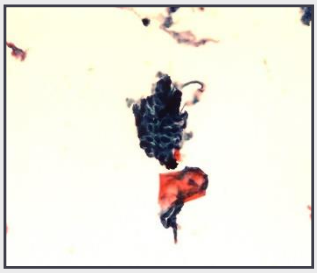
	Histological diagnosis(gold standard)	
	BCC	**AK**
Cytological diagnosis (ThinPrep® Pap test)		
BCC	6	0
AK	0	2
Non-BCC and non-AK(other diagnosis)	0	0
Non-evaluable material	2	3
Total number of slides	8	5

Table 12. Evaluation of diagnosis of BCC using ThinPrep® Pap test with histopathology as gold standard.

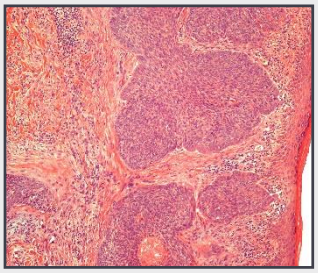
		Histology(gold standard)	
		BCC	Not BCC(AK)
Cytology	BCC	6	0
	Not BCC(AK/non-evaluable/other diagnosis)	2	5
	N, % [95% CI]	Sensitivity: 6/(6+2)=0.75 [0.35-0.97]	Specificity: 5/(5+0)=1.0 [0.48-1-00]

Table 13. Evaluation of diagnosis of AK using ThinPrep® Pap test with histopathology as gold standard.

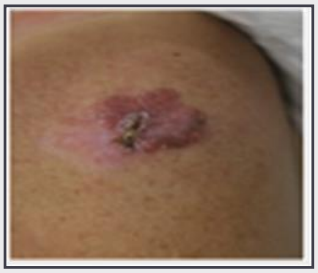
		Histology(gold standard)	
		AK	Not AK(BCC)
Cytology	AK	2	0
	Not AK(BCC/non-evaluable/other diagnosis)	3	8
	Calculation [95% CI]	Sensitivity: 2/(2+3)=0.40 [0.05-0.85]	Specificity: 8/(8+0)=1.0 [0.63-1.00]



Liquid-based cytology showing basal cell carcinoma with atypical small basal cells with scanty basophilic cytoplasm arranged in cluster (x40).

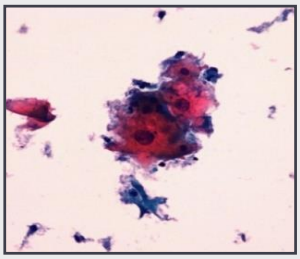


Histology showing nodular basal cell carcinoma with uniform small basaliod cells originating from the basal layer (x10).

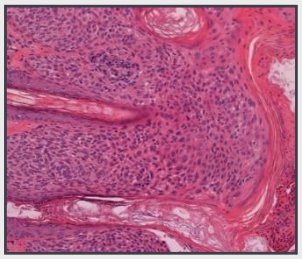


Nodular basal cell carcinoma on shoulder.

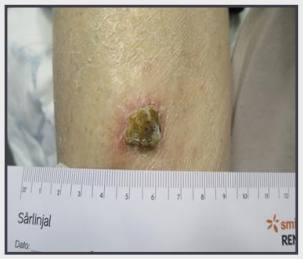
Figure 9. Pictures showing LBC, histology and clinical appearance of the same BCC lesion.



Liquid-based cytology showing actinic keratosis. Atypical squamous cells with uneven contours, and eosinophilic cytoplasm (x40).



Histology showing actinic keratosis with severe atypia. Atypical keratinocytes extending to more than two thirds of the thickness of the epidermis (x10).



Actinic keratosis with hyperkeratosis on anterior calf .

Figure 10. Pictures showing LBC, histology and clinical appearance of the same AK lesion.

DISCUSSION

Altogether 8 BCCs and 5 AKs were included into this pilot study where results show good agreement between LBC and histology in those cases with evaluable samples. No false positive results were found, therefore the specificity was excellent for both BCC and AK. The fact that the investigators knew that the samples histologically were either BCC or AK likely hampered the suggestion of alternative cytological diagnoses.

The CYT-BAK study is to my knowledge the first to report on results of using LBC as a method for diagnosis of AK and BCC. It was designed as a pilot study for initial investigation to assess whether the method may seem useful in such cases. However, the low number of samples included, limits the value of statistical calculations as demonstrated by the wide confidence intervals around the point estimates for diagnostic sensitivity and specificity (Tables 12 and 13). In addition, we found it difficult to include the expected number of lesions into the study as many patients did not meet the inclusion criteria. The main reasons for this were no histology, recurrent lesion, immunosuppressed patient or second time treatment with PDT

The low sensitivity may be a result of the sampling procedure as more than one third of the samples were regarded as non-evaluable. Two BCCs and three AKs were classified as non-evaluable. The histological subgroup of the two BCCs was one nodular and one superficial. In the case of nodular BCC, the cytological sample was abundant in erythrocytes and although using 10% acetic acid before preparation might have resulted in a representative smear, this was not done in this case. The sample of superficial BCC showed a few sheets of keratinizing cells with degenerated nuclei. Both samples of AK were cases of moderate atypia and each showed keratinizing and anucleated squamous cells. One also had a few irregular keratinizing squamous cells, but in general the diagnostic material was too sparse for diagnosis.

An explanation for the relative large number of non-evaluable samples might be: 1) too superficial curettage and/or 2) the cytological brush was too soft for sufficient sampling of viable cells. The use of the Medscand®Cytobrush is gentle to the skin and induces no tissue disfiguration. On the other hand, the soft bristles can make it challenging to sample epithelial cells, especially in tumours of firm consistency. The brush was originally designed for collecting cells from the endocervix for cytological evaluation of epithelial cells. The environment of the cervix is more moist and the tissue softer than the skin. Despite the difference in environment, we found that by rotating the Medscand®Cytobrush across the prepared treatment area, we were able to sample representative cells for cytological evaluation in 8 of the 13 cases. However, we fear that the brush may not be optimal for collecting representative specimens for diagnosis of skin tumours.

In future studies, using a specially designed brush for use on skin may be considered. Such a brush could have slightly stiffer bristles arranged specifically to create a bigger surface area for collecting cells during rotation. Designing such a brush would require engineering expertise, the need of financial resources and time. At the moment this is not a prioritized option. Checking other alternative brushes that are already available is also of interest. Cytological brushes are e.g. used in both endoscopy and bronchoscopy,

alongside cervical cytology. Requested requirements are that they are disposable and that they can be used together with the ThipPrep® Pap test.

Another possible approach for improving sampling results may be to apply tissue from the curette directly to the ThinPrep® container as this may increase the number of representative cells in the specimen. However, a potential problem is contamination with non-diagnostic keratinizing cells.

Sufficient pre-sampling curettage with removal of hyperkeratotic tissue is important. In the present pilot study the curettage technique might have been too gentle as it was chosen mainly to minimize bleeding and further contamination of erythrocytes in the sample. As a result, cells from the deeper layers of the skin may have been misrepresented. In five of our cases, it appeared that curettage did not remove a sufficient amount of keratinizing cells as the samples showed mainly anucleated keratinizing squamous cells.

In my experience, the most important challenges regarding curettage for obtaining representative samples are: 1) bleeding after curettage and 2) the extent/depth of curettage. Deep curettage is beneficial for reaching deeper cell layers of diagnostic cells, but deep curettage can result in more bleeding which reduces the likelihood of attachment of epithelial cells to the preparatory filter. A solution may be to handle all samples with 10% acetic acid.

When preparing bloody samples for evaluation of cells from the cervix using ThinPrep® Pap test, the fixated cells are added 10% acetic acid before preparation. This lyses erythrocytes and thereby increases the likelihood of epithelial cells present on the smear. Blood cells compete with epithelial cells in distribution on the preparatory filter and thus reduce the diagnostic quality of the smears. In the present study, three smears were rich in erythrocytes and sparse in epithelial cells on initial evaluation. The diagnostic quality was therefore not satisfactory in terms of number of epithelial cells. To address this problem, two samples were added 10% acetic acid before final preparation. After applying acetic acid to the relevant samples, we found that more epithelial cells attached to the preparatory filter and as a result the smears showed a greater number of epithelial cells. Moreover, the ability to provide an accurate diagnosis was enhanced. After new preparation and evaluation, the relevant smears showed diagnostic cell groups and a conclusive diagnosis was set. For both samples, the cytological diagnosis agreed with histology. It is therefore beneficial to handle cell samples rich in erythrocytes with acetic acid before preparation. Adding this step to the procedure takes little extra time and does not raise the diagnostic cost noticeably. Moreover, the procedure is a well-known technique used on cervical samples.

LBC is a well-known method for screening of cervical cancer which is both time- and cost effective. It is easier to perform and allows direct fixation of cells after sampling. This results in less artefacts by air-drying, better preservation of cells and better distribution of the cells in the finished sample (8, 9). The ThinPrep® Pap test meets different needs in diagnostic practice, both in terms of quick preparation for prompt diagnostic evaluation, and in cases where instant evaluation is not possible, long durability of harvested samples before fixation. The latter is favourable in cases of long distances between the clinic where the sample is collected and the evaluating laboratory. Moreover, the samples can be stored for some

time and additional evaluation done if necessary. Gynaecological specimens collected using the ThipPrep® container may be stored for up to 6 weeks at temperatures between 15-30° C following collection and prior to preparation (64). In the studies by Onuma et.al (20) and Morrison et.al (15), several advantages were found in the use of LBC. Morrison et.al showed that melanin was more easily detected in smears prepared using LBC in comparison to conventional cytological smears. Onuma et.al found that chromatin patterns and the nuclear membrane was better visualised in LBC compared to conventional Pap. In our study we found that LBC can provide good quality cytological samples, revealing a greater number of cell groups and seemingly giving less background disturbance compared to conventional Pap.

Another point of interest worth discussing is the ability to differentiate between malignant and benign cells by studying the cytological samples. This would be a great advantage in general practice since the cytological test could be used as an initial screening tool for neoplastic skin lesions.

Cytology as a diagnostic method of BCC and AK has limitations; e.g. cytology should not be used when one suspects the lesion to be aggressive in nature. For instance, Morfea type BCC should not be diagnosed exclusively by cytology as it is important to determine tumour penetrance. Also, where morphology is uncertain one should not base the diagnosis exclusively on cytopathological evaluation. Biopsy is often needed to exclude more aggressive types of cancers and to determine whether the lesion should be primarily excised or not (11). Cytology does not give any information about tumour-patterns or subtypes whereas this can be evaluated by histopathology (13, 14). In addition, evaluating the grade of dysplasia/atypia in AK using histology is useful (47). In this study, we were not able to evaluate the grade of dysplasia of AK using cytology, nor subtype characteristics of BCC.

Cytology as a diagnostic tool of BCC and AK is first and foremost favourable in cases where clinical evaluation alone is not sufficient and a non-invasive treatment modality is desired. Using a non-invasive approach in diagnostics supports the desire of an overall non-invasive handling of BCC and AK, which in turn may lead to less psychological stress for the patient and to a favourable cosmetic outcome at the treatment site. Cytology as a technique is also easy, time- and cost efficient (5). The use of cytology in diagnosis of neoplastic skin lesions in transplant recipients and immunosuppressed patients may be beneficial. Transplant recipients are more likely to develop a number of neoplastic skin lesions due to treatment with strong doses of immunosuppressants (38). The use of cytology in diagnostics of such cases is desirable to reduce the number of biopsies necessary for evaluation of the lesions and furthermore to reduce scarring and psychological discomfort to the patient. Moreover, cytological sampling using a brush is time- and cost-efficient. A pilot study on the use of LBC as a diagnostic aid in immunosuppressed and transplant recipients is planned to be carried out in the near future.

My own experience, and information given in pre-existing literature, hold that cytology is a method which is time-efficient, easy, gentle and non-invasive. Furthermore, it leads to no tissue disfiguration, thus eliminating scarring on the site of sampling (5-7, 11, 15, 16, 20). With a cytologist or other trained practitioner present in-clinic, a diagnosis may even be set during the initial visit. Diagnostic cytology may be very favourable when non-invasive treatment modalities are required. The main advantages in choosing

LBC are instant fixation and preservation of fixated cells. Also, the prepared smears show a greater number of cell groups and less background disturbances compared to conventional cytology. Storage of the ThinPrep® container with sample material will not reduce the sample quality. This allows a broad time window in the diagnostic process as samples can be evaluated instantly or at a later occasion and additional cytological evaluations using material from the same liquid container is an option. Lysis of erythrocytes in bloody samples is possible and raises the amount of epithelial cells on the smear.

CONCLUSION

In this study the results suggest that LBC has a too low sensitivity for routine use in diagnosis of BCC and AK. When the cell material was representative a correct diagnosis was made in all cases. Improvement of sampling technique may optimize the diagnostic performance of LBC in NMSC, thus the need for further studies seems appropriate.

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APPENDIXES

Appendix 1 (Ethics approval)

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Gjertil Bergva	22845529	20.05.2015	2015/621 REK sør-øst D
			Deres dato:	Deres referanse:
			24.03.2015	

Vår referanse må oppgis ved alle henvendelser

Eidi Christensen
St. Olavs Hospital

2015/621 Væskebasert cytologi ved BCC og AK

Forskningsansvarlig: St. Olavs Hospital
Prosjektleder: Eidi Christensen

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst D) i møtet 29.04.2015. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10, jf. forskningsetikkloven § 4.

Prosjektleders prosjektbeskrivelse
Basalcellekarsinom og aktinsk keratose klassifiseres som ikke-føflekk hudkreft. Klinisk diagnose bekreftes vanligvis ved histopatologi. Cytologi er en ikke-invasiv diagnostisk metode sammenlignet med biopsi for histopatologi. Der er vist Gode diagnostiske resultat er vist ved bruk av skrapecytologi, men gir opptreden av ikke-diagnostiske uegnede prøver. Væskebasert cytologi kan trolig gi stor grad av representative prøver. Metoden er ytterligere forenklet sammenliknet med skrapeteknikk, men er tidligere ikke benyttet ved hudkreft . Vår hypotese er at væskebasert cytologi gir en høy andel representative prøver og er egnet som diagnostisk metode ved aktinske keratoser og basalcellekarsinom sammenliknet med histologi. Vi ønsker å gjøre en pilotundersøkelse med 10 prøver fra basalcellekarsinom og 10 fra aktiniske keratoser. Cytologisk diagnose sammenliknes med histologisk diagnose (gullstandard). Resultatet kan danne grunnlag for om man senere vil gå videre med liknende, større studier.

Vurdering
Dette er en pilotstudie hvor man skal sammenligne væskebasert cytologi med histologi, som er gullstandarden, ved basalcellecancer og aktinisk keratose. Det skal inkluderes pasienter henvist til Hudavdelingen, St. Olavs Hospital for fotodynamisk behandling av histologiverifiserte basalcellekarsinom eller aktiniske keratoser. Opplysninger som registreres er kjønn, alder, histologisk og cytologisk diagnose, svulstlokalisasjon, størrelse, kvalitet av de cytologiske prøvene. Det er ikke behov for å opprette forskningsbiobank, da prøvene blir destruert etter analyse.

Komiteen har vurdert søknaden og har ingen innvendinger til at prosjektet gjennomføres som beskrevet i søknad og protokoll.

Av søknaden fremgår det at avidentifiserte opplysninger vil bli oppbevart i 10 år etter prosjektslutt. Etter komiteens syn er oppbevaring i 10 år etter prosjektslutt uforholdsmessig lang tid. Komiteen kan gi tillatelse til 5 år oppbevaring av data etter prosjektslutt av dokumentasjonshensyn, jamfør helseforskningsloven § 38. Er det behov for videre lagring utover dette, må det søkes REK om forlengelse.

Vedtak
Med hjemmel i helseforskningsloven § 9 jf. 33 godkjenner komiteen at prosjektet gjennomføres.

Godkjenningen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknad og protokoll, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Tillatelsen gjelder til 01.05.2016. Av dokumentasjonshensyn skal opplysningene likevel bevares inntil 01.05.2021. Forskningsfilen skal oppbevares avidentifisert, dvs. atskilt i en nøkkel- og en opplysningsfil. Opplysningene skal deretter slettes eller anonymiseres, senest innen ett halvt år fra denne dato.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse og omsorgssektoren».

Dersom det skal gjøres vesentlige endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Prosjektet skal sende sluttmelding på eget skjema, senest ett halvt år etter prosjektslutt.

Klagerådgang
REKs vedtak kan påklages, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst D. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst D, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Vi ber om at alle henvendelser sendes inn på korrekt skjema via vår saksportal: http://helseforskning.etikkom.no. Dersom det ikke finnes passende skjema kan henvendelsen rettes på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Finn Wisløff
Professor em. dr. med.
Leder

Gjeril Bergva
Rådgiver

Kopi til: vigleik.jassen@stolav.no
St. Olavs Hospital ved øverste administrative ledelse: post.adm.dir@stolav.no

Appendix 2 (Preparation of samples with ThinPrep® 2000)

Thinprep 2000, preparering av væskebaserte prøver

Forfatter:	Tora Almås, Maj Liv Eide	Versjon:	1.1
Godkjent av:	Gudrun Hovstein Erikstad	ID:	23215
Gyldig fra:	07.12.2015	Revisjonsfrist:	06.12.2017

Hensikt og omfang

ThinPrep® 2000 er et instrument som preparerer væskebaserte celleprøver til celleavtrykk på objektglass for cytologisk vurdering i mikroskop.

Instrumentet kan brukes for å preparere: væskebaserte livmorhalsprøver, urinprøver, bronkiale skyllevæsker, eller finnålsaspirat fra andre organ.

Grunnlagsinformasjon

Se manual for ThinPrep® 2000 og: www.hologic.com / ThinPrep Pap test.

Validering:

Thinprep systemet er validert og CE merket av produsenten (Hologic Inc) for bruk i in vitro diagnostikk. www.hologic.com
Metoden verifiseres gjennom mikroskopering av celleavtrykk etter farging. Metoden er tilpasset og har vært i bruk ved laboratoriet siden 2004.

Ansvar:

Bioingeniører/Cytodiagnostikere ved seksjon for cytologi har ansvar for bruk og vedlikehold av instrumentet.
Se Vedlikehold av ThinPrep® 2000

Fremgangsmåte:

Utstyr:

- Hansker uten talkum
- Linsepapir
- Filterholder
- Filter TransCyt filter
- ThinPrep Slide Objektglass
- ThinPrep prøvebeholdere m/ 30 60% metanol og pasientprøve
- Fikseringsbeholder
- Absolutt alkohol med 2 % MIBK
- Stativ for objektglass og beholder for alkoholfiksering

HMS

Fare-merking	Kjemikalienavn	Avfallshåndtering
Δ	Absolutt alkohol med 2 % MIBK	Eget avløp for etanol på rom 232.04.026
Δ	waste	Eget avløp for etanol på rom 232.04.026

Δ	Thinprep prøvebeholder m/ 30-60% metanol og pasient prøve	Gul smitteboks

Sjekkliste før preparering av celleavtrykk

- Waste flasken må ikke være full (se Max merket). Tømming av avfallsvæske se kap. 5 i manual.
- Påse at hovedmeny vises i displayet.
- Påse at de to O-ringene på filterholderen IKKE er tørre eller krakelerte. Smøring av o-ringer. Se kapittel 5 om vedlikehold.
- Bruk alltid engangshansker under prosessen.

Arbeidsbeskrivelse

1. Åpne døren på instrument ThinPrep® 2000. Se til at prøveholderen og objektglass holderen er tomme, fikseringsbadet skal være fylt med absolutt alkohol. Ta av lokket på prøvebeholder.

2. Sett inn ThinPrep-beholder.

3. Sett inn filter: Ta tak i sidene på et nytt filter med linsepapir og sett den åpne siden av filteret ned på filterholderen. BERØR ALDRI FILTERMEMBRANEN. Sett inn sammensatt filter og filterholder i sporene - se tegning A. Sporene vil bevege seg litt. Ved korrekt innsetting vil filteret henge over prøvebeholder.

4. ThinPrep objektglass merket med Sympathynummer settes inn. Sett glasset inn med skriveflaten til høyre og ned. Korrekt innføring, se tegning B. Ved fjerning av glass, dytt glasset ned i forkant og ta glasset forsiktig ut.

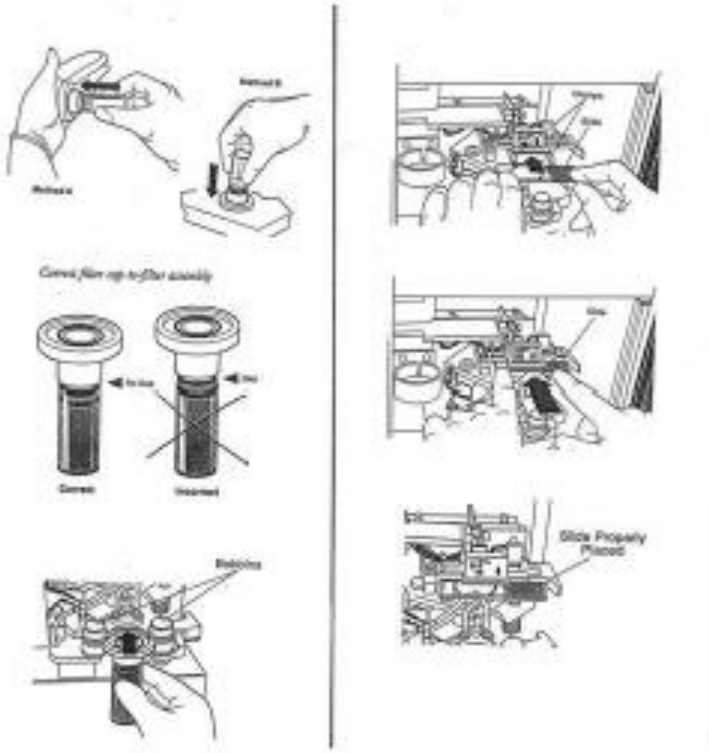
5. Fikseringsbad fylles med absolutt alkohol til nivå mellom MAX og MIN. Skift fikseringsbad etter 100 prøver. Sett badet inn i holderen i instrumentets øverste venstre del.

6. Lukk døren og velg program:
   a. Livmorhalsprøver: Velg program 4 - GYN
   b. Urinprøver: Velg program 2 - FLU/FNA
   c. Bronkiale skyllevæsker: Velg program 4 - GYN

   PS: Urinprøver og bronkiale skyllevæsker skal på forhånd ha blitt overført til ThinPrep Pap Test beholder. Se Bronkial skyllevæske, preparering og /eller Preparering av urinprøver

7. Start prosessen.
   Ikke åpne døren under prosessen. Dette kan medføre tap av celler eller lufttørking. Dersom prosessen må stoppes av en eller annen grunn, se prosedyre i manual kap 3.20.

A. TransCyt filter ***B. ThinPrep objektglass***



8. Ta ut ferdig prøve:
9. ÅPNE DØREN ved å dra den til høyre
10. Fjern fikseringsbeholderen og prøveglasset.
11. Ta opp glasset og sett det til fiksering i ett ståglass med absolutt alkohol. Fikseres i ca 10 min.
12. Ta ut filterholderen med filter, og ta av filteret med linsepapir. Filter er engangsutstyr og kastes sammen med linsepapir i gul smitteboks. Linsepapiret hindrer kontaminasjon fra prøve til prøve via hansker.
13. Ta ut Thinprep prøvebeholder. Skru på lokket.

Prøvebeholder cervix lagres i arkivskap i romtemperatur til prøven er besvart.

Prøvebeholder urin, bronkial eller fra andre organ lagres ca.14 dager i kjøleskapet sammen med ferdig behandlede væskeprøver. Dersom ThinPrep-prøvebeholder for urin eller bronkial skyllevæske er helt tom, skal den kastes i gul smitteboks.

Resultat:

Thinprep celleavtrykk som er klar til farging. Se

Det er referert til en ugyldig dokument-ID: «31887»

Appendix 3 (Papanicoulaou staining method with Gemini AS)

Papanicolaous fargemetode

Forfatter: Tora Ånås, Maj Liv Eide, Shahriar Omidbakhsh
Godkjent av: Harald Aarsæt
Gyldig fra: 18.10.2013
Versjon: 1.3
ID: 14612
Revisjonsfrist: 27.07.2017

HENSIKT

Hensikten med Papanicolaous fargemetode er å synliggjøre cytologisk materiale i lysmikroskop. Fargeresultatet gjør det mulig å vurdere biologisk aktivitet og differensiere mellom ulike celletyper og deres modenhet.

OMFANG

Prosedyren omfatter farging av celleprøver fra cervix og annet alkoholfiksert cellemateriale. Fargeprosessen utføres i Shandon Varistain fargemaskin.

GRUNNLAGSINFORMASJON

PRINSIPP

Papanicolaous fargemetode er en trikrom fargemetode (Fosfowolframsyre = PTA og minst to anionfarger). Det brukes en vannbasert kjernefarge og to metanolbaserte cytoplasmafarger.
Rehydrering av fiksert cellemateriale er nødvendig for at cellene kan ta opp kjernefargen, mens dehydrering forbereder cellene for cytoplasmafargene. Fargemetoden benytter seg av fargemolekylenes størrelse, ladning, fargeløsningens pH og cellenes permeabilitet.
Fargemetoden benyttes for å få frem veldefinert nukleært kromatin, gjennomskinnelig cytoplasma og differensiere mellom keratiniserte og ikke-keratiniserte epitelceller.

Hematoxylin er en kjernefarge. Den aktive komponenten hematein (oksidert hematoxylin) binder seg til PO^{4-} på DNA med Al^{3+} som bindeledd. Hematein-Al^{3+} kalles ett metallkompleks, som binder seg sterkere til cellene enn ionebinding.
Nukleinsyrene farges dersom pH i fargeløsning er > 2 og ideelt rundt pH 3 3,5. Ved for høy pH blir kjernefargen for mørk og ved for lav pH blir fargen for lys.
Hematoxylin brukes progressivt ved at det farges til det er nok og deretter skylles det i en svakt basisk løsning. Dersom springvann har riktig pH, kan dette brukes.
Hematoxylin kan også brukes regressivt. Dette medfører at kjernefargen overfarges og deretter skylles det i en sur løsning, som fjerner overskuddsfarge.

- Hematoxylin farger kjernen blå. Heterokromatin(inaktivt) farges mørk blå og eukromatin(aktivt) farges lys blå. Proteiner i cytoplasma farges blå avhengig av pH.
  - Vurdering av adekvat kjernefarge: Fargen skal være mørk nok til at kromatinstrukturen i Intermediære plateepitelceller er godt synlig, og lys nok til at kromatinstrukturen er synlig i nøytrofile granulocytter.

Orange G (OG) er en cytoplasmafarge. Det er en sur farge (anionfarge), som binder seg ved ionebinding til NH_3^+ i proteiner i cytoplasma. Best fargeresultat med pH 6,5.

- OG farger prekeratin rosa og keratin skarpt orange. Erytrocytter farges fra gulorange til rød.

EA består av anionfargene Eosin og Light Green (inkl. PTA).
EA binder seg ved ionebinding til NH_3^+ i proteiner i cytoplasma.

- Eosin farger superfisielle celler rosa og nukleoler røde.
- Light Green farger biologisk aktive celler blågrønne

Light Green har de største fargemolekylene, deretter kommer PTA, eosin og minst er Orange G. PTA som er et fargeløst molekyl, hindrer at Light Green kommer inn for fort. De minste molekylene går inn i cellen først. Ved lengre fargetid, vil de større fargemolekylene fortrenge de mindre. Det er større permeabilitet hos umodne, aktive celler.

Etter Hematoxylin skylles det med vann, etter OG og EA skylles det med etanol. Det er viktig at skyllebadene skiftes regelmessig, da Hematoxylin ekstraheres i syre (OG og EA er sure farger) og OG og EA ektraheres i vann.

ARBEIDSBESKRIVELSE

Reagenser:

Hematoxylin
OG 6
EA 50
Etanol (abs.alkohol)

Utstyr:

Shandon Varistain Gemini fargemaskin
Rundfilter (185 mm i diameter sort)
Trakt
Kolber

1. Alle fargeløsninger filtreres hver morgen. Fargereagensene byttes ut hver mandag: Dette fordi fargeevnen reduseres og det er anbefalt å skifte farger etter mellom 700 -800 prøver.
2. Farger, vann og alkohol fylles i fargekopper og plasseres i fargemaskinen etter vedlagte program.
3. De cytologiske prøvene plasseres i stativ i rennende vann for rehydrering i 10 min. Dersom sprayfiksativ med carbowax er benyttet, må prøven forbehandles i etanol i 10 minutter før vann.
4. Prøvene settes inn i maskinen i dør A, som er programmert for Papanicolaous fargemetode. Væskebaserte cervixprøver farges med program 5. Konvensjonelle cervixprøver og etanolfiksert annen cytologi farges med program 16.
5. Fargesjekk utføres hver dag på første stativ i PAP og MGG. Ansvarlig bioingeniør for væskelab godkjenner fargene og kvitterer på kalenderen.
6. Første etanolbad etter OG 6 og EA 50 skiftes for hvert tredje fargestativ (ca 60 prøver). De påfølgende etanolbadene skyves frem og et rent etanolbad plasseres sist.
7. Etter siste farging for dagen, helles farger tilbake på respektive brune glassflasker. Skyllebad med etanol helles i avløp merket: xylen og alkohol over 70 %.
8. Farge- og skyllekopper vaskes og skylles for hånd.

ANSVAR

Prosedyren utføres av laboratorieteknikere ved Avdeling for patologi og medisinsk genetikk, seksjon for cytologi ved St. Olavs Hospital. Bioingeniører/Cytodiagnostikere har delt ansvar for fargesjekk og nødvendige endringer i fargeprotokollen.

Appendix 4 (Coverglass protection with Thermo Scientific Microm CTM6)

Bruk og vedlikehold av dekkglassmaskin Microm Cover-Tech CTM 6

Forfatter:	Tora Almås, Maj Liv Eide	Versjon:	2.0
Godkjent av:	Harald Aarset	ID:	16743
Gyldig fra:	29.07.2013	Revisjonsfrist:	23.06.2016

Hensikt

Kvalitetssikre bruk og vedlikehold av Microm Cover-Tech CTM 6 dekkglassmaskin.

Omfang

Bruk og vedlikehold av Microm Cover-Tech CTM 6 dekkglassmaskin.

Arbeidsbeskrivelse

Ansvar

Dekkglassmaskinen brukes og vedlikeholdes av laboratorietekniker og bioingeniører ved seksjon for cytologi, Avdeling for patologi og medisinsk genetikk. Ingeniører ved Medisinsk teknisk avdeling har ansvar for maskinen ved driftsstans.

Plassering

Maskinen står på rom 232.04.026 Lab. for farging og preparering.

HMS

Dekkglassmaskinen har en beholder for løsemiddelet Xylen og en for dekkglasslimet Eukitt, som inneholder Xylen.
Det brukes Xylen/sprit for å rengjøre maskinen.
Det benyttes dessuten dekkglass, som kan knuse og spre glasskår.
Bruk hansker og bytt dem umiddelbart dersom de kommer i kontakt med xylen.
Bruk vernebriller ved rengjøring av maskinen.

Bruk og vedlikehold

Daglig bruk

Slå på maskinen. Strømbryter er til venstre på baksiden. Sjekk, evt. etterfyll Xylen beholder (*fig.2* A) opp til 2/3 deler.
Sett stativ med objektglass i magasinet (C). Teksten UP på stativet må vende opp. Objektglassene skal ligge med etikettsiden opp og etiketten inn mot maskinen.
Trykk YES for å starte (se fig.1 1).
Når stativet er ferdig, piper maskinen én gang.
Stativet tas ut av maskinen. **La stativet stå med objektglassene liggende horisontalt**.
Objektglassene bør så fort som mulig tas ut av stativet, tørkes forsiktig på baksiden og legges på preparatbrett. Dette for å unngå at objektglassene limes fast i stativet.
Dersom det knuses glass i maskinen, må maskinen rengjøres for glass evt. limsøl umiddelbart.
Etter bruk etterfylles limbeholderen med Eukitt dersom det er nødvendig, pass på at det ikke blir for mye bobler i limet.
Slå av maskinen.

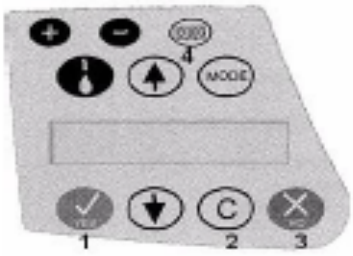


Fig.1

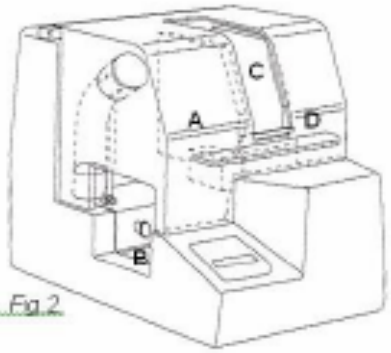


Fig.2

Ukentlig vedlikehold

1. Trykk på NO (3).
2. Ta ut den svarte plata i bunnen av maskinen (B) og Xylen beholderen. Disse rengjøres ved å legge dem i Xylen ved siden av maskinen.
3. Avfallskoppen bak Xylen-beholderen trekkes frem og rengjøres med Xylen/sprit
4. Bruk en finger og tørk av støvet av sugekoppen som flytter dekkglass. Dersom den er tilgriset med lim, vent til limet er nesten tørt før det skrapes av med fingertuppen. **Ikke bruk løsemidler eller skarpe gjenstander.**
5. Vask magasinet med Xylen og rens til slutt med sprit. Bruk bakepensel og cellestoff.
6. Carrier; metallpinner som objektglassene føres på før de pålegges dekkglass, rengjøres med Xylen/sprit.
7. Ta ut dekkglass (D). Rengjør området (bruk Xylen, og tørk av med rent cellestoff).
8. Trykk på pil opp (4).
9. Rengjør resten av området.
10. Rengjør klypa som drar fram objektglass.
11. Legg tilbake dekkglass og etterfyll om nødvendig.
12. Den svarte plata i bunnen av maskinen tørkes over med sprit og legges på plass igjen etter at området der den skal ligge er rengjort (bruk Xylen/børste).
13. Xylen-beholderen og området rundt der den er festet rengjøres (bruk Xylen). Beholderen fylles med ny Xylen, opp til ca. 2/3 av beholdere, før den settes på plass.
14. Om nødvendig etterfylles limbeholderen med Eukitt (opp til streken).
15. Trykk på C (3) og følg instrukser på display. Trykk på C igjen og maskinen er klar til bruk, eller den kan slåes av.
16. Signer på vedlikeholdsskjema.

Denne prosedyren finnes i papirformat ved fargemaskinen.

Appendix 5 (Diagnosis and classification of cytological samples)

Registerings nummer: ☐☐

Væskebasert cytologi ved BCC og AK

Cytologisk diagnose: BCC ☐ AK ☐ Annet ☐ Ikke diagnostisk ☐

Kommentar:

Dersom annet, forslag til diagnose:

Uegnet pga blodtilblanding: Ja ☐ Nei ☐

Ny vurdering etter lysering av blod: Ja ☐ Nei ☐

Antall celleflak/grupper i preparatet:

Relativ kjernestørrelse: N/C ratio:

Kromatin mønster: Nukleoler:

Bilde tatt: Ja ☐ Nei ☐

Histologisk diagnose: BCC ☐ AK ☐

Vurdert/dato: Underskrift:

Appendix 6 (Consent form for participation in study)

Væskebasert cytologi ved BCC og AK 220315

Forespørsel om deltakelse i forskningsprosjektet *«Bruk av væskebasert cytologi ved diagnostikk av basalcellekarsinom og aktinisk keratose»*

Bakgrunn og hensikt

Dette er et spørsmål til deg om å delta i en forskningsstudie for å undersøke om celler tatt fra overflaten av hudforandringer der legen mistenker hudkreft kan brukes diagnostisk. Pasienter som allerede har fått påvist forandringer med diagnosen basalcelle kreft eller aktinske keratoser og som er henvist til Hudavdelingen ved St. Olavs Hospital for fotodynamisk behandling vil bli spurt om å delta i studien.

Dersom legen mistenker hudkreft er det vanlig å ta en prøve som sendes inn til undersøkelse for å bekrefte diagnosen (histologisk undersøkelse). Ofte vil dette være en liten hudprøve fra hudforandringen som tas med en liten kniv. Det er også mulig å ta andre typer prøver for å bekrefte diagnosen ved visse typer hudkreft. Hudoverflaten kan skrapes lett med en kyrette (skrapeteknikk) og celler som skrapes fra hudoverflaten blir undersøkt. Dette vil være en cytologisk undersøkelse. Cytologisk undersøkelse ved skrapeteknikk har i tidligere undersøkelse vist seg å være god i diagnostikk av basalcelle kreft og aktinske keratoser. En enda mer skånsom prøvemetode er væskebasert cytologi. Det er spesielt denne prøvemetoden vi nå ønske å studere. Ved denne metoden brukes en børste til å få med seg celler fra det syke hudområdet som så overføres og oppbevares i en væske før cellene farges og materiale undersøkes. Cytologisk diagnose sammenliknes med histologisk diagnose. I denne studien håper vi å få et bilde på om væskebasert cytologi kan egne seg i diagnostikk ved basalcellekarsinom og aktinske keratoser. Cellepreparatene vil bli fotografert med tanke på formidling av kunnskap og publisering

Studien er et samarbeidsprosjekt mellom en student, leger/forskere og ansatt ved St. Olavs Hospital og NTNU, under ledelse av overlege/ forsker Eidi Christensen (St. Olavs Hospital/ NTNU). Studien vil inngå i en hovedoppgave for medisinerstudent ved Det medisinske fakultet, NTNU. Veiledere vil være professor, overlege Christina Vogt, seksjonsleder Maj-Liv Eide og Eidi Christensen.

Det er St. Olavs Hospital som er studieansvarlig institusjon.

Hva innebærer studien?

Du er allerede henvist til fotodynamisk terapi av hudforandring som enten er et basalcellekarsinom eller aktinsk keratose. I forkant av fotodynamisk behandling blir behandlingsområdet bedøvet med lokalbedøvelse og deretter skrapes for å fjerne skorper og hard hud. Dette gjøres rutinemessig for at medikamentet som benyttes bedre skal kunne trenge ned i huden. Det vi ønsker å gjøre i forbindelse med studien er å ta prøver fra overflaten av huden etter fjerning av skorper og overfladisk hard hud. Celleprøvene tas med en børste som trykkes lett ned mot hudoverflaten for å hindre blødning og roteres mens den føres over området to ganger. Prøvetakingen tar under 1 minutt. Cellemateriale overføres så til en væskebeholder og transporteres til cytologisk seksjon på sykehuset for nærmere undersøkelse. Noe av cellematerialet som ble skrapt bort med kyretten vil bli støket ut på et objektglass, for undersøkelse. Deretter forløper den fotodynamiske behandlingen som normalt.

Mulige fordeler og ulemper

Du mottar lokal bedøvelse og deretter skrapes/kyretteres behandlingsområdet som ledd i forbehandling før PDT. Ut over vanlig prosedyre for fotodynamisk behandling vil du kun utsettes for bruk av den cytologiske børsten som fanger opp celler i det allerede skrapte området. Børsten roteres forsiktig i vevet for å forhindre blødning. Vi vurderer dette til ikke å påføre deg ubehag eller bivirkninger. Vi håper resultatet av studien peker i retning av at væskebasert cytologi kan

egne seg som diagnostisk metode for visse typer hudkreft og derved danne grunnlag for at liknende, større studier kan bli gjennomført på et senere tidspunkt.

Hva skjer med prøvene og informasjonen om deg

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste

Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Celleprøvene destrueres etter analyse. Datamateriale slettes i 2026.

Det vil ikke være mulig å identifisere deg i resultatene av studien eller i fotografiene av cellene når disse publiseres

Frivillig deltakelse Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte:

Dr. Eidi Christensen, Hudavdelingen, St. Olavs Hospital på telefonnummer: 72822065

Ytterligere informasjon om studien finnes i kapittel *A* *– utdypende forklaring av hva studien innebærer.*

Ytterligere informasjon om personvern og forsikring finnes i kapittel B *– Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring følger etter kapittel B.

Kapittel A- Hva innebærer studien?

Hensikten med studien er å vurdere om kvaliteten på utstryk fra basalcellekarsinom (BCC) og aktinisk keratose (AK) preparert ved hjelp av væskebasert cytologi ThinPrep®Pap, er tilstrekkelig god for bruk i diagnostikk og om væskebasert cytologi synes velegnet som diagnostisk metode ved BCC og AK sammenliknet med histologi.

BCC og AK inngår i samlebetegnelse ikke-melanom hudkreft, og utgjør den vanligste form for kreft hos mennesker med lys hud. Histopatologisk undersøkelse av prøver fra hudsvulster er vanlig å ta for å verifisere klinisk diagnose. Imidlertid er cytologi en ikke-invasiv metode sammenlignet med biopsi for histopatologisk vurdering. Metoden er enkel, skånsom, kostnadseffektiv og raskere å utføre. Cytologi med skrapemetode har vist seg velegnet som diagnostisk metode for visse typer av hudkreft dersom andelen av representative prøver er tilstrekkelig for diagnostikk. En svakhet med metoden er imidlertid opptreden av ikke-diagnostiske uegnede prøver, der det cytologiske utstryket i hovedsak viser keratiniserte celler eller celledebris.

I denne studien ønsker vi å se på resultat og kvaliteten av prøver ved bruken av væskebasert cytologi som diagnostisk metode. Væskebasert cytologi kan trolig gi stor grad av representative prøver sammenliknet med bruk av skrapemetoden, da alt cellemateriale blir overført til prøvebeholder, eliminasjon av lufttørking gir godt bevarte celler og cellene blir jevnere fordelt med mindre overlapping enn ved skrapeteknikk. Parvise cytologiske og histopatologisk prøver fra individuelle BCC og aktinske keratoser vil bli undersøkt og resultatene sammenlignet.

Pasienter som er henvist hudpoliklinikken, St. Olavs Hospital for fotodynamisk behandling (PDT) av allerede histologiverifiserte, primære (AK) og (BCC) vil bli forspurt om å delta i studien. Ekskludert er gravide, ammende, immunsupprimerte og barn under 18 år. Likeledes er BCC av histologisk morfea type ekskludert. Cytologiske prøver ønskes fra 10 BCC og 10 AK etter standard forbehandling for PDT. Det tas cytologisk prøve fra kun en lesjon hos hver pasient. Pasienter som deltar i studien får gjennomført sin fotodynamiske behandling som planlagt. Som ledd i standardprosedyre vil behandlingsområdet bedøves ved lokalbedøvelse og deretter skrapes overfladisk for å fjerne skorper og hard hud. Dette gjøres for at medikamentet (krem) som

benyttes bedre skal kunne trenge inn og ned i huden. I studiesammenheng vil en cytologisk prøve tas fra behandlingsområde etter lokalbedøvelse og skraping. En cytologisk børste vil trykkes forsiktig ned mot huden og roteres mens den føres over området to ganger. Prøvematerialet på børsten overføres raskt til ThinPrep® Pap test prøvebeholder og deretter transporteres det til seksjon for cytologi, Avdeling for patologi og medisinsk genetikk, St. Olavs Hospital for videre preparering og farging. Den cytologiske vurderingen blir utført av en cytodiagnostiker og en patolog. I tillegg vil noe av cellemateriale som ble fjernet fra lesjonen med kyrette i forbindelse med skraping bli støket ut på et objektglass, fiksert og farget for undersøkelse.
Da den cytologiske børsten roteres forsiktig i vevet for å forhindre blødning og føres kortvarig over det allerede bedøvede og preparerte området, vurderes denne prosedyren til ikke å påføre deg ubehag eller bivirkninger.
Dersom du velger å delta, vil det ikke medføre noen ekstra kostnader for deg. Du vil ikke motta noen form for betaling for å delta i studien.

Kapittel B - Personvern, økonomi og forsikring

Personvern

Opplysninger som registreres om deg er er kjønn, alder, histologisk og cytologisk diagnose, svulst lokalisasjon, størrelse, kvalitet på cytologiske prøver.
Representanter fra de ansvarlige for studien (Dr. Christensen/ Dr. Vogt/seksjonsleder Eide og student Isham) og kontrollmyndigheter kan få utlevert studieopplysninger og gis innsyn i relevante deler i din journal. Formålet er å kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal. Alle som får innsyn har taushetsplikt.
St. Olavs Hospital, Trondheim ved administrerende direktør er databehandlingsansvarlig.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert.
Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi

Studien har ikke legemiddel- eller har ikke annen ekstern finansiering.

Forsikring

Du er forsikret gjennom Norsk pasientskadeerstatning

Informasjon om utfallet av studien

Så snart studien er avsluttet vil resultatene publiseres, gjøres offentlig tilgjengelig. Du kan ta kontakt med ansvarlige leger etter at studien er avsluttet for informasjon om resultatene

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien, og gir tillatelse til at fotografier av cellepreparat kan benyttes i kunnskapsformidling og i publikasjoner.

(Signert av prosjektdeltaker, dato)
Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix 7 (Consent form for inclusion of photo material)

Samtykke til taking og bruk av bilde(r)

Jeg, ... født ..... samtykker til at det kan bli tatt bilde av min hudforandring og at bildet kan benyttes i undervisningssammenheng, i faglige presentasjoner og i publikasjoner. Ved bruk av bilde vil ingen personidentifiserbare opplysninger bli gitt.

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Dato	Pasientens underskrift
.....	.....
Dato	Legens underskrift