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Methodology article

Virtual in Vivo (Predictive) Autopsy: From knowledge and understanding to education, research and communication in digital tissue – based diagnosis.

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Abstract

Background: The digital world is entering all compartments of tissue – based diagnosis, especially education, training and performance of surgical pathology.

Theory: Communication is a requirement of life. It is based upon knowledge, understanding, and adequate response. Understanding tries to implement and spread concordant or target related actions. Analysis of liquid biopsy, cytology, biopsy, surgical specimens and autopsy comprise the tissue – based sources. They are transferred into images and create the basis of education and training, followed by research and publication.

Present Stage: Liquid biopsies require the automated application of digital tools, such as digital visualization and statistical analysis of the obtained DNA / protein figures. Manual interference does not occur.

Cytology, aspiration smears, biopsies, and surgical specimens are still fixed, processed in conventional manner, and placed on glass slides. Digital microscopy replaces conventional light microscopy in some pathology institutes. It is usually applied close to its analogue performance. Diagnosis assistants are used for quantification of specific image features, for example to score the expression of functional cellular markers. Digital microscopy is an important compartment of the available Hospital Information System.

At present, autopsies do not contribute to tissue – based diagnosis in a notable frequency. Even big University Pathology Institutes report an autopsy frequency less than 100 cases, in comparison to approximately 100,000 biopsy specimens or even more per year. Most authors name live imaging investigations (CT, MR, Ultrasound, etc.) for reason. An additional factor might be the diminishing impact of understanding in medical diagnostics: Highly precise information of individual (small) tissue compartments is frequently considered to be sufficient for treatment. They include receptor expressions, intra-cellular pathway

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abnormalities, gene alterations, etc. This seems to be a contradiction to 'organ communitive information' obtained from autopsies. Such post mortem information can also be obtained during the patient's life time and predict the probable trails of recovery or death by use of digital pathology. The procedure is called 'in vivo' or 'predictive autopsy' and described in detail herein.

Future aspects: Digital pathology is entering the field of 'automated diagnosis', starting with automated recognition of 'regions of interest' and associated characteristics such as automated diagnosis, digital self - recognition, automated failure repair, treatment advises, etc.

The field of 'digital autopsy' will remain reserved for education because of need for 'real autopsies'.

The proposed 'in vivo (predictive) autopsies' offer additional perspectives of digital tissue – based diagnosis, which include the digital analysis of tissue / organ dysfunctions and syntax at life time, and the impact on forecast the recover /disease progress of the patient.

Conclusions: Digital pathology is on its way to enter numerous implementations of tissue – based diagnosis. We propose digital 'in vivo (predictive) autopsies' as a new tool to analyze, explain and forecast the involvement of all organs in the individual patient's disease development, and to interpret the 'cause of death' more in detail.

Keywords: digital pathology, in vivo (predictive) autopsy, tissue – based diagnosis, liquid biopsy, life imaging.

Introduction

Tissue – based diagnosis is a reliable, frequently applied medical procedure to detect, analyze, forecast and treat nearly all human diseases [1-3]. It comprises the analysis of human body structures at different magnification levels. These range from the appearance of the whole body via organ features down to subcellular pathways and macromolecules [1-3]. The arrangement has been named 'orders of structures', and reflect a basic property of the majority if not of all structures in nature [4-6].

Our knowledge of presentation and actions of life has been notably increased in our 'digital times' at all levels, ranging from 3D formations of macromolecules to behavior of various living populations [7-9]. Most if not all of this knowledge is electronically available to man, independently of education and place of residence [9]. In addition, specialized expert conferences transfer the most recent available

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knowledge to interested persons either in physical or virtual participation [1, 2, 10]. Detected features of an individual structure or function do not include all information of the analyzed process, and do not allow to derive all features of higher or lower ordered structures [1, 5].

In image analysis, any detected structure is equivalent to an object, the spatial (or time related constellation) to a new, higher ordered structure [2, 11-13]. Higher ordered structures can either be investigated directly or computed, if the spatial / time serial arrangement of the lower ordered structures is known [3, 4]. The algorithm requires the spatial distribution of the objects and a neighborhood condition, for example the most frequently applied Dirichlet's tessellation (Voronoi's neighborhood condition) [10, 14].

The spatial algorithm can be expanded to a time – related algorithm, which permits to forecast the fate of the analyzed structure [6, 12]. Often, the concept of entropy permits reliable and reproducible (transferrable) results, especially in cancer diagnosis [6, 12]. Most of these investigations have been performed at the cellular (biopsy – surgical specimen) level [3].

Today, liquid biopsies replace some, if not all clinically wanted diagnosis / information of cytology and / or biopsy, just using a different level of structures (circulating macromolecules) to describe and forecast the impact of the object sources on the fate of the whole system (man) [15-21].

Herein we describe the 'order' of applied tissue – based diagnosis investigations in man, and its potential performance in digital pathology. They include in increasing order liquid biopsies, cytology, fine needle aspiration, biopsies, surgical specimens, and autopsies.

Autopsies are rarely investigated and have lost most of their attraction in our days. They stay in the focus of this article, because they belong to the roots of pathology, and digital pathology offers some interesting aspects to collect information from autopsies which may not be assessable otherwise.

Tissue – based diagnosis - history & basic aspects

Tissue – based diagnosis, which include all medical diagnoses that are based upon investigations on human tissue range back into prehistoric times. Stone age man performed trepanations of the human cranium and probably investigated the brain / skull tissue. Ancient Greece and Chinese body drawings and medical reports indicate that some information of organ location and function has been detected. In the medieval autopsies were performed to collect information of organs and

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diseased compartments. They served for knowledge, understanding of death, medical education and diagnosis. Pathologists, who performed autopsies operated as surgeons and dentists too.

The 'break through' in tissue analysis occurred in the 19th century, when new microscopes permitted a detailed insight in tissue structures and their activities [22-26] [27].

From that time tissue – based diagnoses served for communication standards between pathologists and clinicians. The diagnosis names were derived from both morphology (small cell lung carcinoma) and clinical behavior (bronchitis, tuberculosis, etc.).

Today, the meaning of 'diagnosis' is changing from the cellular – based descriptive 'advice to the clinician' (small cell lung cancer might not be treated by surgical intervention) to alterations of macromolecules which might forecast of the individual patient's disease development [28-30]. The technology includes investigations of genetic, epigenetic, and environmental abnormalities [16, 31, 32]. It strongly depends upon the technical accuracy and to some aspect on accidental and time related unavoidable influences, such as noise, chaos, fluctuations, etc. [9, 16, 31-33].

Performance and Impact of digital tissue - based diagnosis

1. Liquid biopsies can be considered a 'follow – up technique' of search and analysis of circulating abnormal blood compartments (tumor cells, CTC), which started to be clinically applied for cancer diagnosis in the 1990s [21, 34-37]. The development of advanced molecular biology techniques such as amino-acid sequencing, macromolecule magnetic resonance imaging and Raman spectroscopy permitted a sensitive verification of circulating tumor cells and their intra-cellular compartments [16, 18, 20, 31]. The accuracy and sensitivity mainly depend upon the cell type and less upon localization and size of the lesion [38]. Live imaging techniques such as computerized tomography (CT), nuclear magnetic resonance imaging (NMR) and scintigraphy can accurately investigate and measure both localization and size of the lesion. A combined investigation of liquid biopsies and live imaging informs about the diseases (genetic) nature (for example cancer) and its precise localization and extension [34, 36, 39]. Most clinical applications have been reported from lung cancer, followed by lymphomas and breast cancer [15, 19, 34, 36, 39]. Cardiovascular diseases are also subject for liquid biopsies as well as rare cancer cell types such as ocular cancer [37, 40, 41]. All these investigations require digitized

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biochemical, molecular genetic / epigenetic data. Digital tissue – based diagnosis is mandatory to perform and interpret liquid biopsies [35, 37, 42].
2. Cytology, fine needle biopsies and surgical specimens are still fixed and processed in conventional manner [10, 43-46]. The aim of diagnoses derived from this material (morphology) is to name and classify the disease; in other words, to assign potential clinical treatment too [47-49]. The diagnosis appoints to different targets of clinical actions, such as complete recovery, long survival, quality of life, or detection of recurrence. Several diagnosis features need already quantitative or semi-quantitative measurements, which include tumor size, inflammatory respond of host tissue, tumor cell grading, or proliferation rate [45, 50]. Digital tissue – based diagnosis permits accurate and reproducible measurements of cells and tissue, especially staining intensities, gene abnormalities, and configuration at different magnifications [12, 13, 46]. Artificial or automated tissue cut sequences are used to reconstruct 3-D images [49, 51], which permit an insight into the 'real' appearance of the lesion [49, 51].

The main aim of digital pathology derived from cells and tissue remains equal to that of conventional performance. Its advantages include easy and fast communication with clinicians, direct implementation into the Hospital Information System, automated and reproducible intern quality control, fast and comprehensive retrieval, accurate measurements and observance of molecular configurations [45, 49, 52].

3. Autopsies still belong to education and training of medical doctors who want to become a surgeon or pathologist. All Western European Countries report difficulties to provide young colleagues with a number of autopsies to sufficiently fulfill the legal requirement. The actual rate of autopsies amounts to only 5% - 10% of that in the 1980s [28, 46, 53]. They are not attractive for young colleagues from the standpoint of training or from that of research. In addition, they are not paid well, if at all. Therefore, several authors suggest 'to replace' real autopsies by virtual autopsies [54-59]. These investigations usually take the cadaver and CT examinations to visualize the post mortem condition of the organs [60-66]. The CT investigation or NMR visualization are consistent with and do not alter legal evidence of the organs, which can be re-used for additional physical examinations. Performed CT examinations can be combined with additional NMR imaging and with physical biopsies taken from the detected lesions [54, 55, 67-70]. Most virtual autopsies of adult cadavers were performed due to forensic legal reasons, in contrast to virtual autopsies of still births and fetal malformations [58, 71-76].

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In aggregate, virtual autopsies consist of virtual imaging of the cadaver and contemporary analysis of physical cytology, fine needle and biopsy specimens, which may be analyzed virtually too [54, 55, 62, 69, 77, 78]. Physical autopsies investigate in the localization of organs inside the body, inspect the individual organs and take (large) biopsies for microscopic, genetic and molecular pathology analysis. In non – forensic cases they focus on the proof the treatment of the dead person during her / his life time, and to investigate in the so – called cause of death [58, 70, 73, 79, 80].
4. Virtual in vivo (predictive) autopsies aim to analyze, describe and forecast the development of a disease of a living patient. They are composed of CT, NMR, Scintigraphy and Ultrasound images and physical tissue – based diagnosis. A time sequence related algorithm computes the obtained data on the basis of physiological interactions of the organs. Vascular, lymphatic vessels, and nerves create the 'inner' connective transport ways, as well as the upper and lower respiratory tract, gall ducts and intestines represent the connective pathways within the person's environment. In addition, the male and female reproductive system as well as eyes and ears might be included. A general scheme of this algorithm and the included different orders of structures including the general predictive significance are exemplarily demonstrated in <Figure 1>
The scheme is arranged to the different orders of structures, which start at the macromolecule (level 1), and are terminated at the 'organ' (level 7). An open one way direction of the 'communicative pathways' is common; however, it may be occluded or replaced by several either contemporary or continuous flows in contrary or not completely overlapping directions, for example in case of cancer, inflammation, or inborn abnormalities. Live images (CT, NMR, Ultrasound, etc.) inform about the position, surface and internal structures of each organ. Naturally, overall or focally distributed organ functions may become visualized too. In aggregate, the proposed in vivo (predictive) autopsy includes virtual anatomy in combination with organ dysfunctions / lesions ascertained by tissue – based diagnosis procedures at different life times of the patient. Their goal includes the understanding of the individual's health stage at the date of in vivo autopsy and its probable development. It permits an understanding of the 'cause of death' from a 'communicative point of view'.

Discussion

Virtual anatomy sections are well developed and have been introduced in medical education since approximately 1990 [81-87]. They inform about the location and size

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of organs, there normal physiologic conditions, and their actions [82, 85, 88, 89]. Several accurate programs are open to be downloaded in the internet. Some of them include sophisticated animation [86, 87, 89-93].

Virtual autopsies are different. They are aimed to explain the diseases of an individual case, and do only crudely refer to the basic human body. Therefore, a virtual transfer of the body's individual reality requires different and more complicated algorithms.

In microscopy, for example, the 'normal appearance and classification' of cells can be demonstrated in one example only. It refers to all cases in the past and in the future, because changes of 'normal appearance' will not occur, or are not expected to happen.

The virtual transfer of a cell population in a diseased organ can only be verified by thorough measurements of the cellular features and reproducible association of the features with the corresponding cell type. Thus, the 'pathology' or 'autopsy algorithm' is of different, and hard to implement nature compared to that of its 'anatomy' equivalent.

Until today, virtual autopsy is understood as practical tool to 'avoid' the 'opening' of the corpse and to substitute the otherwise not assessable information by radiologic / resonance images in combination with tissue analysis of the 'lesions (organs) of interest' only. The procedure of virtual autopsy is equivalent to stratified sampling under the microscopic e [50], to the search for 'hot spots' in some malignancies, or to detect 'regions of interest' in virtual microscopy [1, 2, 7, 94]. The strategy transfers the 'random sampling' (analysis of a 'biased – free' sample of objects which are present in the measurement plane /order) to a 'higher' plane /order [9, 12, 29, 49, 95]. Such an algorithm has to possess and use 'own knowledge' or 'self-recognition' at a low grade if it should run automatically [7-9, 96]. Virtual anatomy sections do not possess this property, because all of them stay at the same 'grade of knowledge', independently from their implementation or animation features.

The proposed 'in vivo (predictive) autopsy', however, will possess some different grades of 'autonomous' knowledge, for example the automated recognition of organ lesions, and its association with the 'normal' appearance in the body, as well as the 'automated' estimation of time – related changes, and their projection in the future or back to the 'start conditions'.

At later stages it will become a promising tool to analyze and investigate in the communication pathways of an organ or body. These will not only communicate at

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the same level ('order of structures') but also interact between different levels. Concrete predictive measurement parameters will probably include entropy calculations, distance measures, and time series analyses. All of them have been reported to allow a reproducible description of structures and functions of human organs and to forecast probable organ alterations and failures [4, 10, 29, 45]. In this context 'in vivo autopsies' may mature to a new tool for both the patients to get familiar with the limitations of life and the medical experts to stratify diagnosis and treatment at different organ / body compartments.

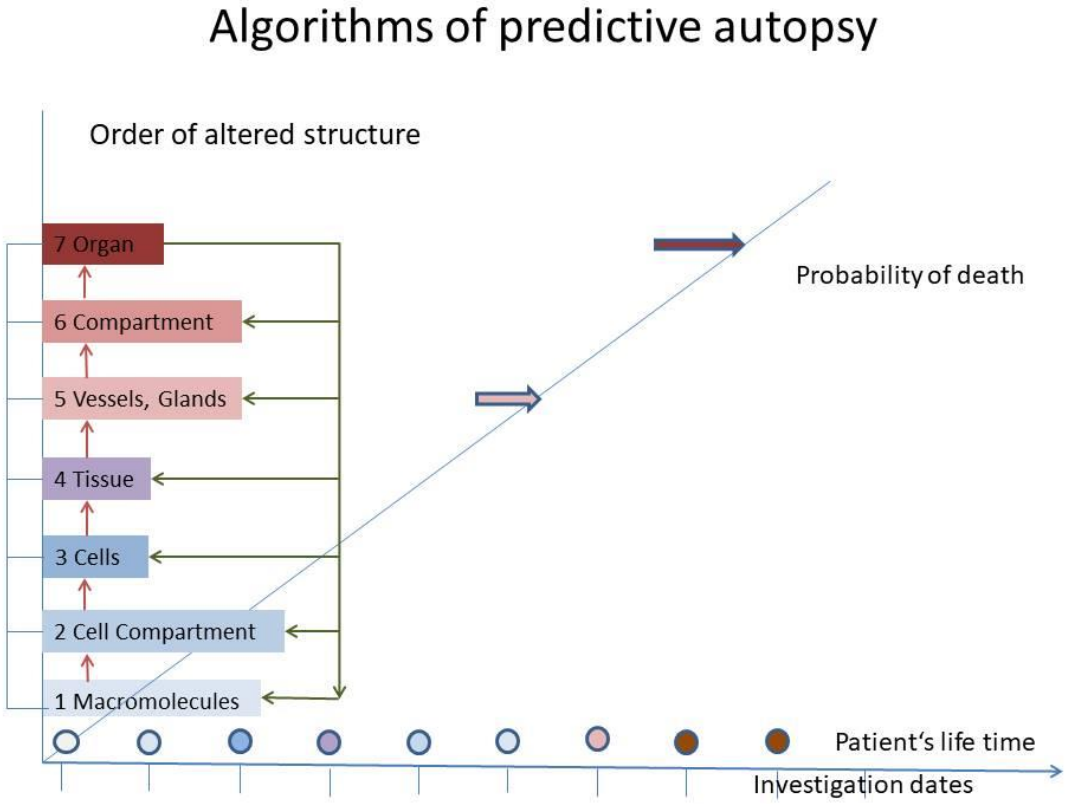

Figure 1: The vertical axis represents the affected order of structure, the horizontal axis the patient's life time and the dates of investigations. A linear association between the involved structures and the forecast of life time is assumed. The natural involvement of different structure levels (jump to the next higher level) is shown in red, that of potential external actions (repair) shown in green. Additional factors such as severity, activity and internal interactions (feedback, immunoreaction, etc.) as well as the reversibility / non - reversibility of lesion (cancer, inflammation, infection, infarction, etc.) are not included in this scheme of the principle algorithm. Practical implementations have to take into account (weight) additional parameters, such as localization and size of the lesions as well as age and sex of the patient.
It should be noted, that the altered structure may be recognized / identified by virtual assessment (for example high resolution CT), and do not necessary require physical examination. Antique Chinese map of an abdomen (according to Yang Ki-Tscheu) [5].

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