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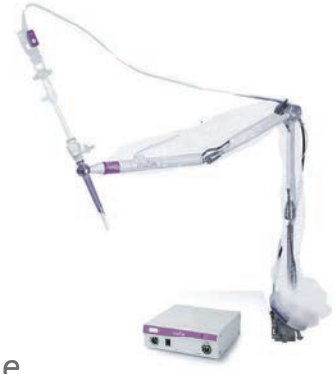

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Editorial

Aleš Tomažič
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The Section for Endoscopic Surgery was founded as part of the Slovenian Medical Association in 1991, and in the same year the first endoscopic surgery congress was held under the leadership of the association's first president, Vladislav Pegan. On this basis the section was renamed, and the Slovenian Society for Endoscopic Surgery was founded in 2009. After many years, the society almost discontinued all of its activities, and in 2017 we were at a crossroads in deciding whether to terminate the society's activities or to build a new basis for future development of the society. We chose the the second option: a new leadership was elected, connections with neighboring countries were established, and our position within the European Association for Endoscopic Surgery (EAES) also started to grow.

In 2017 we had 30 members, and now, by 2022, our membership has increased to more than 60. All our members are also members of the EAES. The EAES is a strong professional society with 3,300 members mainly from Europe, but also from some other countries. Our work in EAES was also recognized with the nomination of a member to the Education and Training Committee.

During this period, we successfully held our 14th congress with international participation, which took place in Portorož in 2018. In 2019 the society launched a new journal, *Surgery and Surgical Endoscopy*, and since then two issues have been published every year. Thanks to both of its editors-in-chief, Jan Grosek and Tomaž Jagrič, the journal is well accepted

among Slovenian surgeons, and we hope that in the future, with the internationalization of the editorial board, growth will also be achieved internationally. A significant share of our members are young surgeons, and we have put great effort into holding at least two workshops a year for them (open and laparoscopic surgery workshops).

With the upcoming congress in Bled, which will take place from May 26th to May 28th, 2022, a new president will be elected. I am sure that our society will grow further in all directions and, as its past president, I will assist as much as possible.

Aleš Tomažič

Editorial

Abbott

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1

Indikacijo za prehransko podporo postavi zdravnik na oddelku (kirurg ali prehranski tim), na podlagi prej postavljene diagnoze za kirurško zdravljenje oziroma ocene o stanju prehranjenosti bolnika z metodo NRS2002.[1]

2

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3

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Human Neural Tissue Sources and Surgical Sample-Collecting Methods

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neural tissue, neurosurgery, cell culture, cell isolation, tissue harvesting

Review Article

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Abstract

The techniques of cell isolation supported by modern laboratory procedures have been rapidly progressing, offering a wide range of in vitro research opportunities. The first and one of the most important steps in cell isolation is tissue harvesting, which is most commonly performed during surgical procedures. This is the basis that determinates the success of isolation. This article describes human neural tissue sources and neurosurgical approaches to tissue collection.

Introduction

During the last decades, cell biology has been rapidly evolving (1). New laboratory methods, instruments, and experimental conditions offer numerous research possibilities for cell cultures, which represent a suitable alternative to animal and human experiments (2). With cell culture techniques, cells can be isolated from tissues and maintained in culture, where suitable conditions for their growth and proliferation are provided. Their range of use in preclinical and clinical medicine is wide, in addition to other areas of research, such as experimental pharmacy and the pharmaceutical, cosmetics, and food industries (3–5).

The modern development of cell cultures started in the early 20th century with Harrison and Carrel, who were the pioneers of the cell culture techniques. They formulated the methods to study cellular function in vitro, separately from the influences of other factors in the body. In 1907, Harrison studied nerve fiber growth in frog embryo cell culture, and Carrel isolated and maintained chicken heart muscle cells in 1912 (6, 7). These cell isolation techniques were quite simple at first. The tissue was cut into small pieces and partially digested enzymatically. Under experimental conditions, the cells then migrated from these tissue fragments and proliferated, forming a cell culture. Preserved only in saline, these tissue samples were the basis for cell isolation (8). They were harvested during various surgeries and

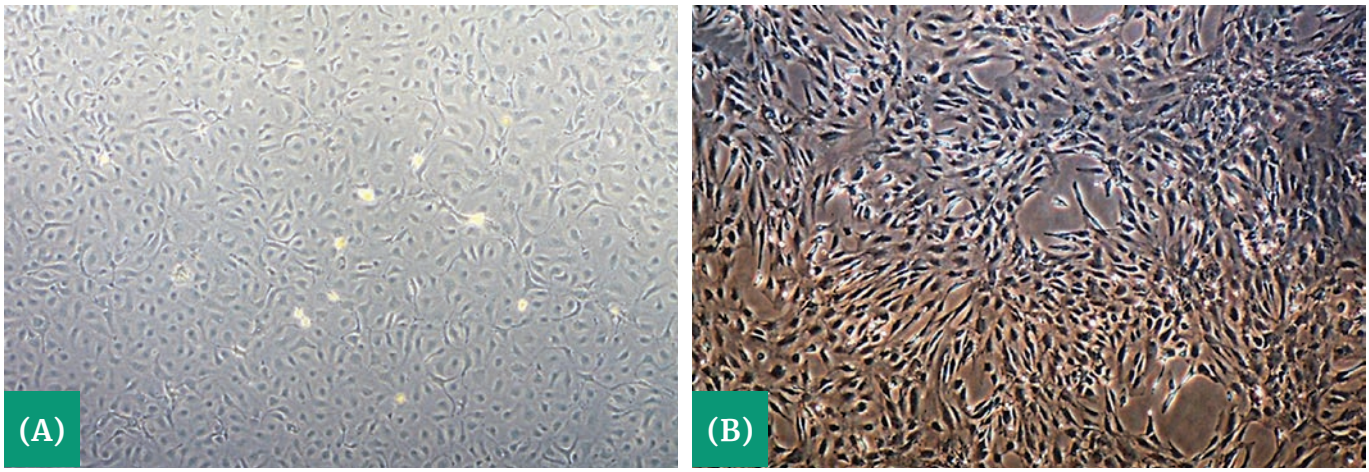

Figure 1. An example of cell isolation from a healthy adult brain. **A)** The primary culture of human astrocytes in a low-density culture. Individual polygonal cells are evident. Images were taken at ×50 magnification on a Zeiss Axiovert 40 inverted microscope. Scale bar = 200 µm; **B)** Neoplastic cells from a human brain. The glioblastoma cells were isolated from the resection specimen. A characteristic polymorphic cell appearance is evident with scant cytoplasm and various shapes of cell nuclei. Nicon Diaphot 300 inverted microscope. Scale bar = 100 µm.

quickly transferred to the laboratory. This isolation technique prevailed for about half a century until other more advanced techniques were developed and were modified for specific tissues and experimental conditions (9, 10). Today, cell isolation techniques have become a routine practice in research laboratories in the medical and pharmaceutical sciences (9, 11, 12).

Different tissues are sensitive to various degrees to harvesting, transport, and storage before laboratory processing begins (13). Neural tissue is particularly sensitive. Incorrect harvesting and handling may lead to sample damage, which is reflected in the cell isolation yield and cell growth in culture (14). Neural tissue can be harvested during various neurosurgical procedures and is used as a source for different types of isolations, both normal cells and tumor cells (15, 16). Because of their role in epilepsy, neurotrauma, Alzheimer's and Parkinson's disease, dementias, multiple sclerosis, and tumorigenesis, their isolation is of paramount importance. For successful cell isolation, however, it is also necessary to consider the surgical technique of tissue harvesting (17, 18). The sources and neurosurgical approaches to neural tissue collection for cell isolation are briefly described.

Neural Tissue Sources for Cell Isolation

There are many tissue sources for neural cell isolation. Due to interspecies differences, animal cells cannot be directly translated to study similar processes in humans, despite their ready availability, easy accessibility, and reasonably uncomplicated maintenance in cell culture (15, 19, 20). This makes human neural cells more desirable for research. The sources for human neural cell isolation include neonatal and adult brain and spinal cord (Figure 1). The tissue may be normal or neoplastic, depending on the experimentation purposes (19–21).

In comparison to neonatal brains, tissue for isolation is much more readily available in adults, both in quantity and in the frequency of harvesting. Neonatal brains may be obtained during elective abortions from fetuses, usually 9 to 12 or 22 weeks of age (20). The timing for tissue collection is of vital importance, and therefore good cooperation between the clinical department and the laboratory must take place. Not all fetuses are suitable for isolation. Only neural tissue from fetuses collected in vacuum aspirations can be used for culturing. When abortion was performed after a medical procedure, the tissue is not suitable because the pharmaceutical agents for fetal death may alter

cell viability and consequently impede the growth characteristics of the primary culture (20, 22, 23). In adults, on the other hand, neural tissue is harvested during gross resections in open surgeries and in various kinds of biopsies (Figure 2). Adult brain tissue is taken from the cortex in patients undergoing craniotomy for trauma, tumor, epilepsy, and vascular surgery (19, 20, 24, 25). Trauma-derived tissue is one of the most frequently used types. Some limitations may exist here, however. Care must be taken to use tissue from the edge of the resected specimen and not to include contaminated and necrotic tissue, which elevates the risk of contamination (20, 24). Similarly, specimens directly from the penumbra, where the tissue is damaged but still viable, are best avoided (24, 26). In addition to trauma, a variety of nervous system disorders, such as metabolic diseases (hyperammonemia and hypoglycemia), ischemia, hypoxia, and epileptic seizures, go together with neuroglial cell damage, consequent difficulties in growth of the cell culture, and reduced number of passages (20, 27). In tumors, necrotic parts should be avoided, and only viable neoplastic tissue should be collected. In unruptured aneurysm surgery, which is an elective operation, a tiny part of brain tissue is removed from the aneurysm dome. This tissue has not been affected by pathologic conditions, as in epilepsy surgery, where the tissue is abundant and histologically intact. During brain biopsies for deep-seated brain lesions, deep brain structures are accessible, such as the hypothalamus, insula, and basal ganglia. All these specimens are excess brain tissue, usually small and more fragile, making them prone to desiccation and autolysis when not properly stored (20, 21, 23, 28).

Because neurosurgical operations can occur at any time and unpredictably, the timing, method of tissue transfer to the laboratory, and availability of trained laboratory staff are very important (29). Reagents and storage facilities must be at hand. The time and method of tissue transfer to the laboratory may vary and are very important. In neonatal brain samples taken during abortions, the tissue is more problematic due to its fragility and instability. The transfer time is typically less than 2 hours (29, 30). In adults, on the other hand, the tissue is usually more stable because it is taken during biopsies and resections, and it reaches the laboratory much more quickly. At our clinical department, the tissue transfer time is up to 20 minutes (Figure 3). Because adult brain tissue is easily accessible and abundant due to the larger number of surgeries in comparison to neonatal sources, it is the preferable tissue source for experimentation (31, 32).

Neurosurgical Approaches for Brain-Tissue Sampling

With the advent of new technological possibilities, surgeries are becoming less invasive for patients (33). The type of neurosurgical procedure depends on many factors, such as tumor location and size, vascularity and composition, multiplicity of tumors, accessibility, the eloquent areas of the brain, the clinical condition and wishes of the patient,

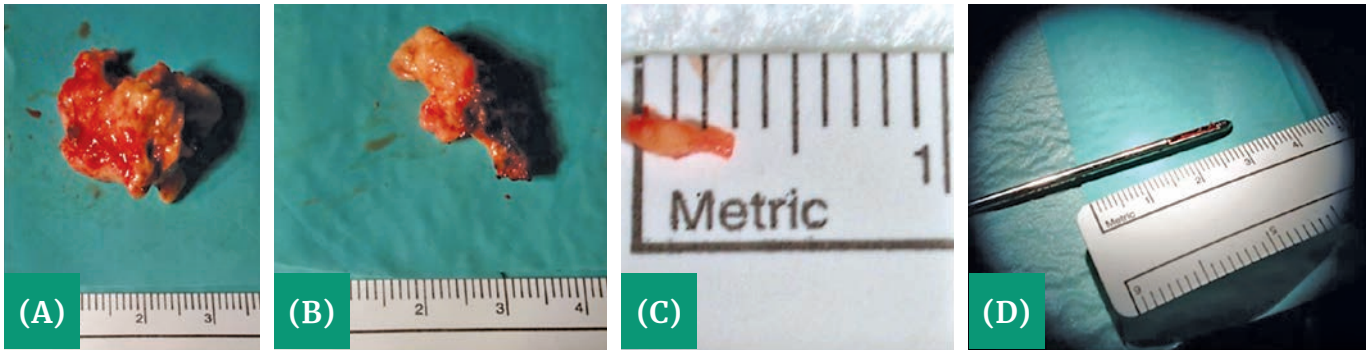

Figure 2. Various resection specimen obtained in brain surgeries. **A)** In open surgery and in gross resections, abundant tissue is available. It can be used for further processing in the cell laboratory, as in open glioblastoma surgery; **B)** The resection specimen of glioma obtained from open biopsy or the keyhole approach, which is smaller; **C)** The glioma sample in needle biopsy; **D)** The biopsy needle with the tissue sample.

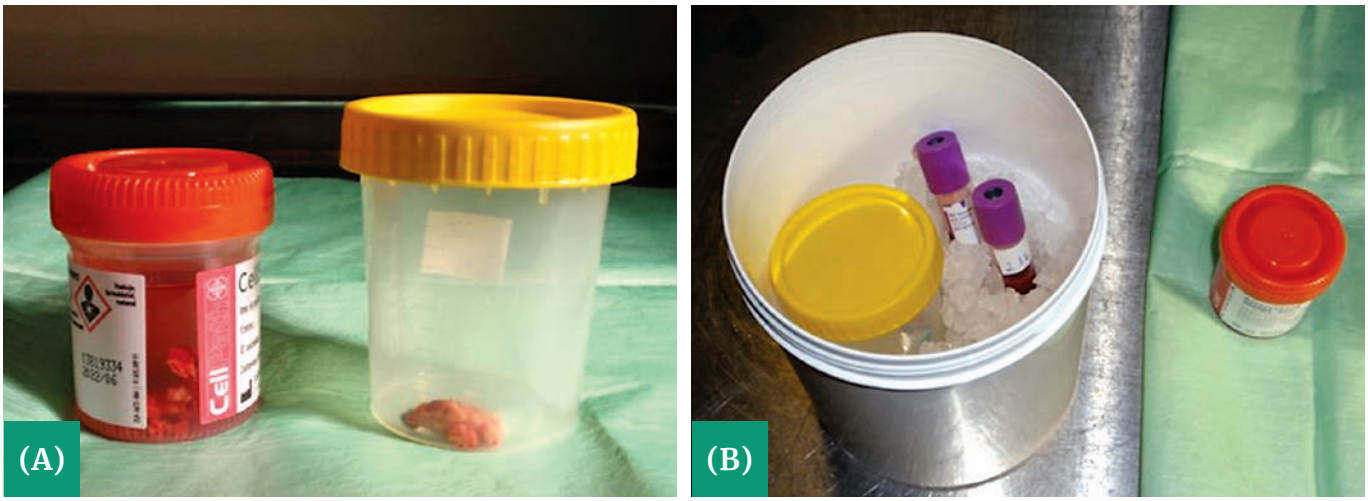

Figure 3. After resection, neural tissue samples are prepared for transport. **A)** A glioblastoma specimen is directed to the cell laboratory for cell isolation and to the pathology department for histopathological evaluation. The pathology-destined specimen is conserved in formaldehyde solution (left) and the tissue for the cell laboratory will be transported in saline (right); **B)** The aim is to transport the tissue on ice and as quickly as possible to reduce cell death.

and the surgical equipment (34). In addition, from the researchers' point of view, with technical advances and surgical opportunities, the possibilities for acquiring ideal tissue samples are also increasing. They offer possibilities for taking brain-tissue samples from an increasing number of neurosurgical pathologies and from various locations that are now accessible with minimal possible morbidity using easier and less invasive techniques, thus contributing to a higher cell yield during the isolation procedure in the laboratory (35, 36). It is important to note that the treatment aim, which includes the preservation of the neurological function of the patient, is always the first and most important, and that acquiring tissue for cell isolation is in second place. Thus, all surgical samples obtained are excess brain tissue that is not used for further diagnosis. In addition, it is necessary to obtain ethical approval and consent from the patient and family before any experimental manipulation with the tissue (37). There are numerous neurosurgical approaches used in everyday clinical practice that provide a welcome source for both healthy and diseased brain tissue (36, 38).

Open Surgery

With its modifications, open surgery is most frequently used for brain tumors, as well as for all traumatic brain injuries and for most vascular pathology (37). It offers good visibility, accessibility, and reduction possibilities for large tumors, alleviating intracranial pressure and improving neurological symptoms (Figure 4). Superficial lesions are especially suitable for open resections, as well as highly vascularized lesions, for which stereotactic techniques are contraindicated (39). Microneurosurgical instruments must always be available, and the operative microscope offers the best possible visibility. Intraoperative cortical mapping can be used in eloquent areas to limit potential surgical damage (34). Tumor removal by open surgery will also offer information regarding the extent of the involvement of nearby structures and help reduce the sampling errors faced in stereotactic biopsy. In the case of vascular pathology and trauma, open surgery offers good access to the severed vessel and the possibility of brain decompression and necrectomy, respectively (34, 38, 40). With open access, the harvested tissue samples are mostly abundant, and the tissue can be generously used for cell isolations, separating the non-viable parts and using only the most suitable ones.

Typical open neurosurgical approaches involve pterional, bifrontal, convexity, interhemispheric, and suboccipital craniotomies. Orbitozygomatic and transsphenoidal craniotomies are less frequently used, and they provide improved access to the skull base. Decompressive craniotomy for traumatic brain injury is a special entity, offering large exposition of brain areas and

potential necrectomy in damaged regions, and relieving the refractory increased intracranial pressure (40, 41). Craniotomy for superficial tumors typically involves the entire tumor area, whereas deep tumors may be accessed through smaller craniotomies because the intracranial operative field enlarges with increasing distance from the surface (42). The working corridor through the brain parenchyma must be maintained with retractors, using either spatulas or by applying intermittent retraction with handheld instruments during the bimanual manipulation of the tumor. The transcortical approach to the lesion is replaced with a trans-sulcal or trans-fissure approach whenever possible to shorten the corridor (36, 43). The eloquent areas on the surface and also the subcortical tracts and vessels are studied preoperatively using digital tractography imaging, functional MRI, and high-resolution MRI to obtain a safe vector for the working corridor (34, 36, 43). The surgical complication rates range from 2 to 9%. There is a 1.5% mortality rate, 1.5% wound infection rate, and 4.5% hemorrhage occurrence at the operative site. Although most patients show improvement in their performance status postoperatively, surgical morbidity after tumor resection has been reported at 9% (44, 45). Postoperative medical complications may occur in 3–9% of patients. Of course, trauma patients and those with vascular emergencies have a higher risk of complications, depending on the clinical presentation at admission and the extent of the brain insults (37, 45).

Keyhole Approaches

Craniotomy can also be performed through a very small opening or so-called keyhole approach (46). This is a modification of conventional craniotomy and is useful for minimizing approach-associated morbidity, especially for primary tumors (Figure 5). In contrast to conventional craniotomies, keyhole craniotomies are much smaller than the lesions (47). They still make it possible to appreciate the local anatomy and are valuable mostly for deep-seated tumors. Because less anatomy is revealed on the surface, image-guidance is usually necessary and adds to the operative safety (48). Keyhole craniotomies have been effectively and safely used to carry out both biopsies and gross total resection for primary and secondary brain tumors. However, they are not convenient for surgical treatment of brain trauma and edema due to the limited accessibility and small areas exposed (46, 47). The simultaneous use of an endoscope may improve the extent of the resection by accessing the residual tumor in the lateral aspects of the resection cavity, which is not visible with a standard operative microscope (47). Neuronavigation can be used as an adjunct to plan the most optimal surgical trajectory to the lesion. Intraoperative ultrasound or intraoperative MRI, when available, is also helpful (49, 50). Neuronavigation can be omitted in emergencies, especially when the time needed for the preparation may prolong the surgery. As with open surgery, the harvested tissue samples are generous, especially in gross total resections. The factors that influence the tissue quantity and its availability for the laboratory include the accessibility, location, and properties of the lesion (46, 51).

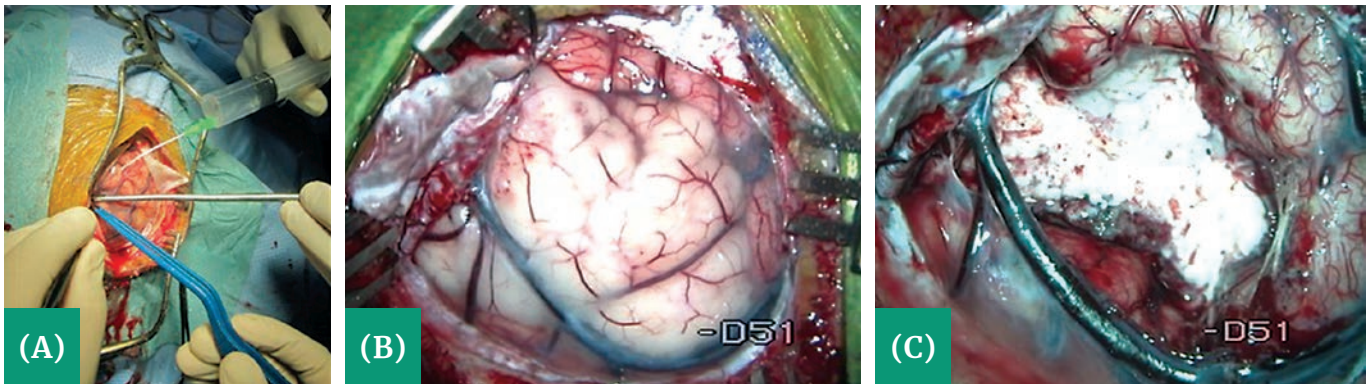

Figure 4. A) Brain exposure in open glioma surgery; **B)** The primary brain tumor is clearly visible on the surface. The tumor tissue is more swollen and whitish-tan; **C)** The resection cavity after tumor removal. The drainage vein below the tumor was left intact because it is important to preserve normal brain drainage

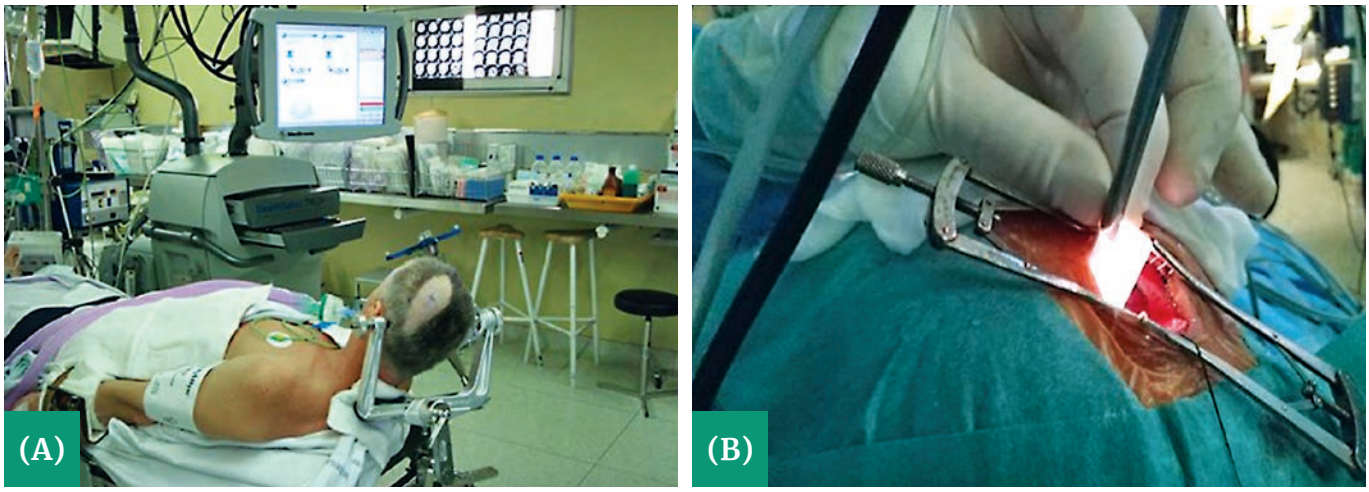

Figure 5. Keyhole craniotomy for primary tumors, which is a modification of conventional craniotomy. **A)** The patient is positioned and the neuronavigation is prepared for guidance. Because less anatomy can be revealed on the surface, image guidance is imperative; **B)** The introduction of the instruments for tumor resection through the keyhole craniotomy.

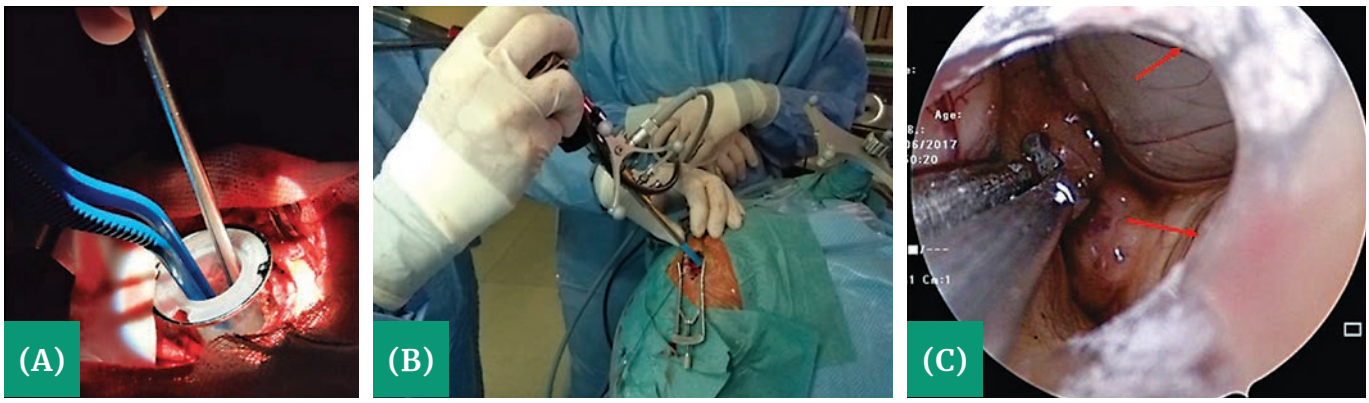

Figure 6. A) A neuroendoport in position, providing a tube-shaped corridor through the brain to deep-seated lesions. The instruments are introduced through the neuroendoport to the lesion with visualization through the operating microscope; **B)** Alternatively, an endoscope can be used, which is introduced here through an expandable neuroendoport. The neurosurgical instruments will follow next; **C)** Intraventricular tumor resection through an expandable neuroendoport. The arrows indicate the lower edge of the neuroendoport corridor.

Neuroendoport Surgery

A neuroendoport is a cylindrical or tube-shaped retractor system that is used as a corridor to deep-seated brain lesions (Figure 6). It allows bimanual surgery with microsurgical instruments under endoscopic or microscopic visualization (52). It is especially valuable for approaching deep brain lesions located in the ventricles and in the basal cisterns, the posterior thalamus, the basal ganglia, and the pulvinar. Before neuroendoport use, such deep lesions were reachable only by stereotactic needle biopsy or with retractor systems and neuronavigation (53, 54). Despite allowing good visualization of the operative field, blade retractors, in contrast to a neuroendoport, may cause pressure on the brain parenchyma, resulting in hemorrhage and vascular injury–induced ischemia. The advantages of a neuroendoport include more evenly distributed pressure onto the walls of the operative corridor, minimizing retractor-induced injury (52, 52). Modern neuronavigation systems and diagnostic imaging have improved the precision of targeting these lesions, as well as operative safety (55).

The neuroendoport technique has become a new standard in the resection of various brain tumors, such as glioblastomas, astrocytomas, ependymomas and papillomas, neurocytomas, gangliogliomas, intraventricular meningiomas, cavernous angiomas, brain abscesses, intraparenchymal hematomas, massive hematocephalus, metastasis, colloid cysts, and choroidal arteriovenous malformations (56, 57). The early reports have demonstrated that minimally invasive endoscopic guided surgery through a neuroendoport is both effective and safe (57, 58). The resection specimens are usually well preserved and copious with both microscopy- or endoscopy-combined neuroendoport surgery compared to those in open or keyhole surgery, thus allowing a sufficient amount of tissue for cell isolation (17, 55).

Stereotactic Needle Biopsy

Stereotactic biopsy is a relatively new technique that was first introduced into clinical practice in the 1970s (59). The aim is to target a very small area or volume in the brain accurately by means of a predefined minimally invasive trajectory. The location of the target is determined according to the reference system, which is composed of various extra- and intracranial markers (60). Stereotactic biopsy can be performed with or without stereotactic frame placement, which acts as an external reference and a coordinate system (Figure 7). A preoperative CT or MRI of the patient's head with a frame mounted and image merging is required in frame-based stereotaxis. On the other hand, frameless systems (image-guided biopsy) are gaining popularity be-

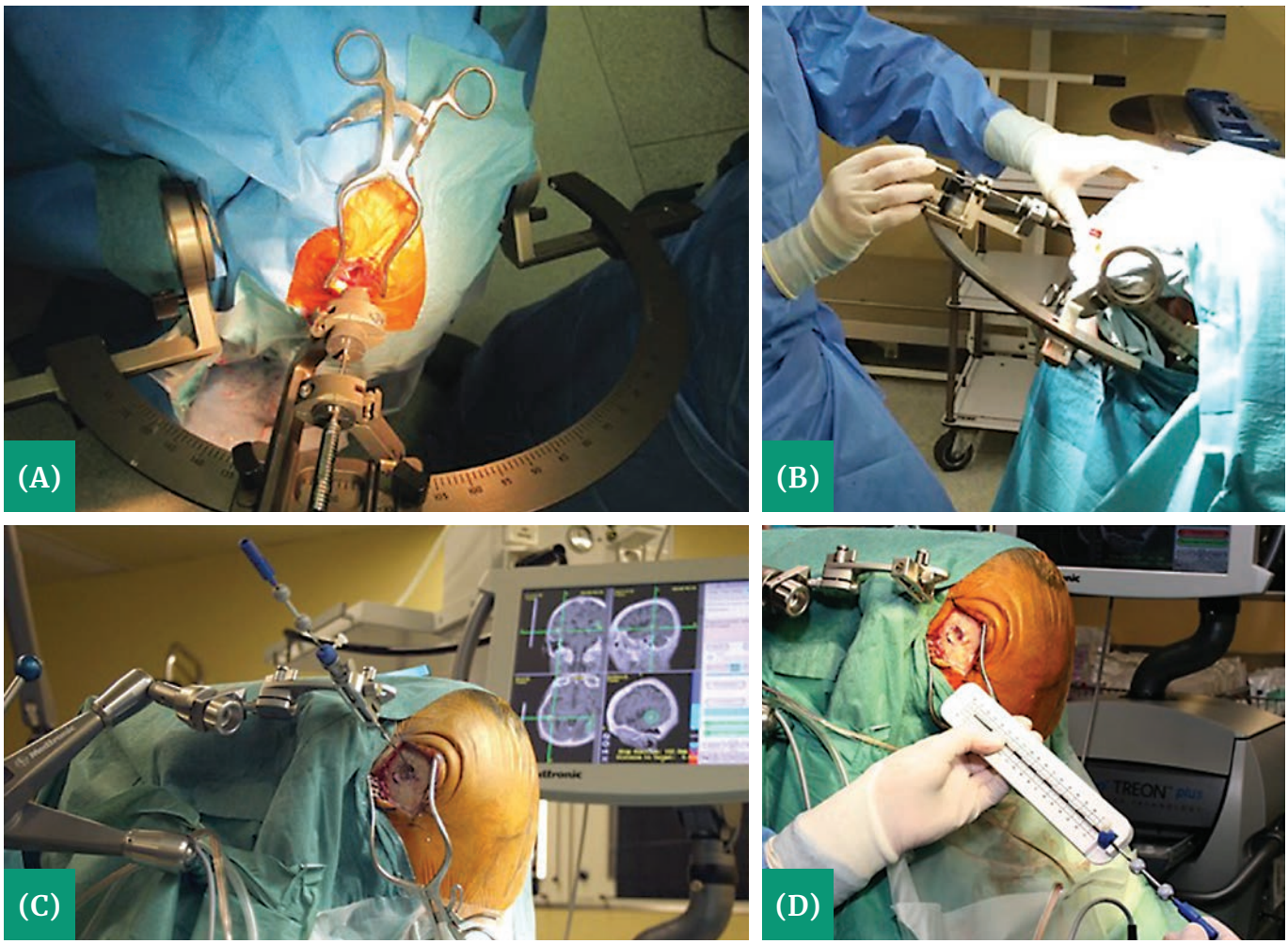

Figure 7. A) Frame-based stereotactic biopsy for deep-seated brain lesions. The stereotactic arch with the attached biopsy guidance introductor is visible; **B)** Introduction of the biopsy needle; **C)** Frameless stereotactic biopsy. The trajectory is adjusted during the operation according to the neuronavigational panning; **D)** The biopsy needle for frameless stereotactic biopsy and needle length adjustment.

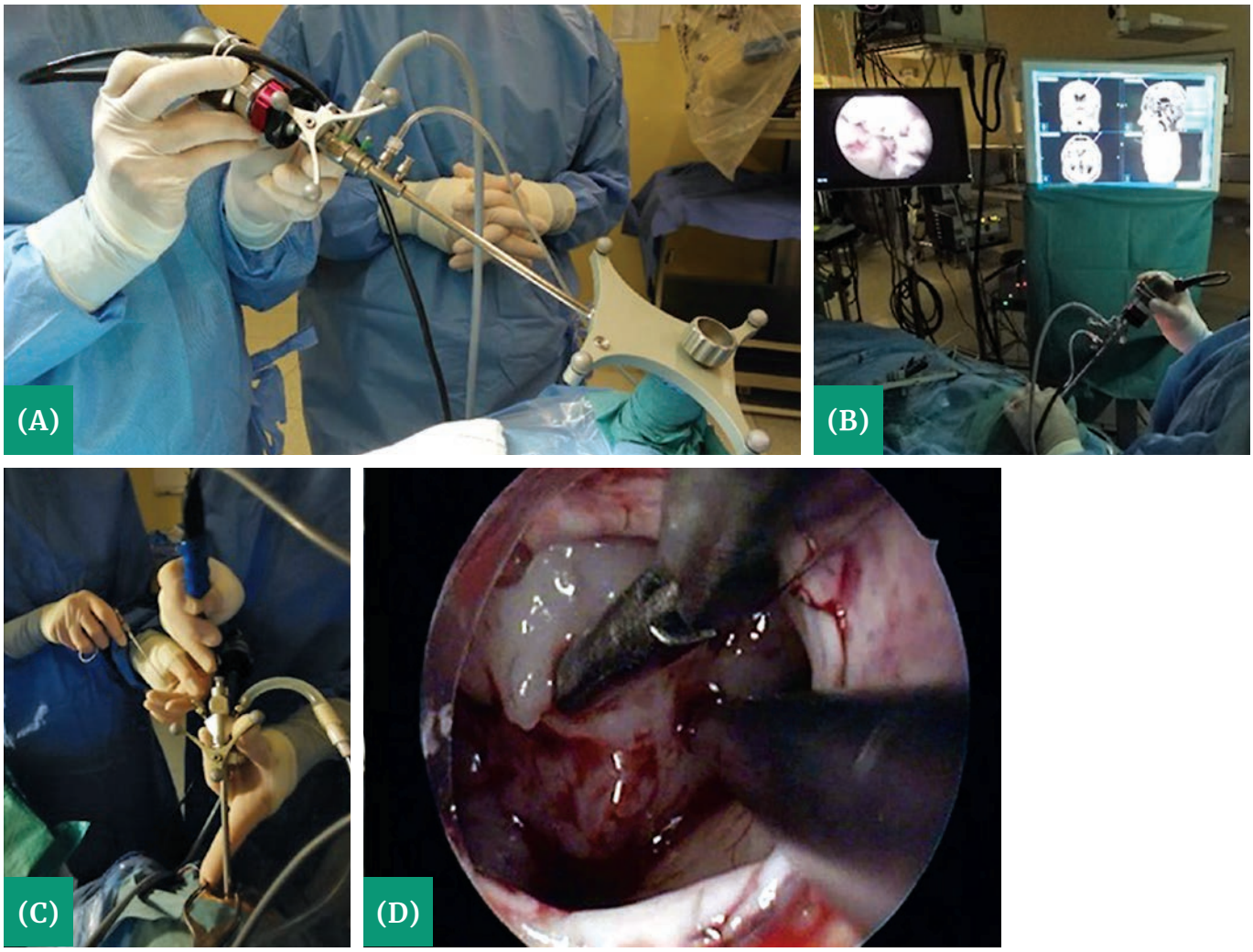

Figure 8. Neuroendoscopy. **A)** First, the neuroendocoscope is navigated; **B)** During surgery, the operative field is observed on the monitor. The exact tip location is controlled from the second screen, which is coupled to the neuronavigation system; **C)** Full endoscopy employing two working channels; **D)** The endoscopic view during tumor resection.

cause their use is easier and faster, and only one preoperative imaging, usually by MRI, is needed. A computer-generated three-dimensional model of the patient's head and brain (from MRI or CT scans) is registered to the patient's actual head position by surface registration of orbitonasofrontal area in the anesthetized patient, which serves as a patient-specific reference. However, some deeply located lesions in the brainstem and posterior fossa can only be precisely and safely accessed with frame-based cranial systems (60–63).

A stereotactic biopsy may provide an accurate pathological diagnosis with a target accuracy of 2–4 mm. The diagnostic accuracy is high, ranging from 70% to 93% (63, 64). It can be augmented by intraoperative fluorescein use, which enhances the diagnostic yield and improves the operative safety. A neurologically intact patient with small, deeply located solitary or multiple lesions with a minimal mass effect is a good candidate for such management, as are patients with cystic tumors that can be drained by aspiration through the stereotactic needle. Highly vascularized lesions are not suitable for stereotactic biopsy. It should be noted that a stereotactic biopsy can be used solely for tumors and other neurodegenerative lesions, and it is more diagnostic than a curing procedure (65–67). The mortality ranges around 2% and surgical morbidity around 3% (68). The tissue samples in this procedure are small, usually about 1 mm in width and 2 mm or 3 mm long, and they may sometimes be heavily contaminated with blood. The size and composition of the tissue

specimen plays an important role in cell isolation because it can affect the number and growth characteristics of the isolated cells. Small tissue samples may also result in sampling errors due to tissue heterogeneity (69).

Neuroendoscopic Surgery

Neuroendoscopy uses an endoscope for the treatment of diverse pathology of the central nervous system (Figure 8) (70, 71). The technique dates back to the early 20th century and has developed greatly ever since. Initially, neuroendoscopic procedures were limited to the ventricles (ventriculostomy). Today, navigated neuroendoscopy is utilized for treatment of a wide range of intracranial pathology inside and outside of the ventricles, in endoscopic suturectomy in scaphocephaly, for implantation of radioactive seeds, and as an adjunct to microscope-based procedures (56, 71, 72).

Neuroendoscopy is a minimally invasive technique with the aim of decreasing approach-related brain trauma and increasing visualization of the tissue through better magnification and illumination (71, 73). The skin wound, craniotomy, and brain exposure are minimal, as is the brain retraction. Full-endoscopic surgery can be effectively used in ventricular pathology (74, 75). Access is provided through the neuroendoscope working channels, usually one or two. Simultaneous use of two instruments makes possible some tissue manipulation. Endoscope-assisted surgery, on the other hand, uses the neuroendoscope solely as a visual aid instead of the operating microscope. The instruments are positioned aside the endoscope and bimanual manipulation with the microsurgical technique is possible (74, 76). Neuronavigation can be used with neuroendoscopy for selection of the optimal burr-hole or neuroendoport position and to choose the safest trajectory to the lesion, thus reducing the risk of damage to the vital structures (56, 77). In addition, in endoscope-assisted surgery the endoscope may be used as an addition to conventional microscopic surgery for final inspection of the resection cavity, offering an angled view (72, 78). The tissue specimens obtained during neuroendoscopy vary in size, depending on the type of the neuroendoscopic method (i.e., endoscope-assisted or full endoscopic). Those obtained with the former technique are better preserved and more abundant because the tissue fragments taken during the full-endoscopic approach must fit the instruments that slide through the tight neuroendoscopic working channel. Consequently, they are small and frequently crushed. This may sometimes hamper successful cell isolation due to a higher number of necrotic cells (76, 79).

Conclusions

The type of neurosurgical approach depends on many patient- and tumor-related factors and ultimately affects the quality of the harvested tissue, which is important not only for the final diagnosis and treatment options, but also for the emerging research possibilities. In addition to better treatment opportunities for patients, novel technological achievements in brain surgery also simplify the collection of tissue neural tissue from various locations, with increasing precision, preservation, and tissue quantities. The integrity and condition of the tissue sample forms the basis for cell isolation.

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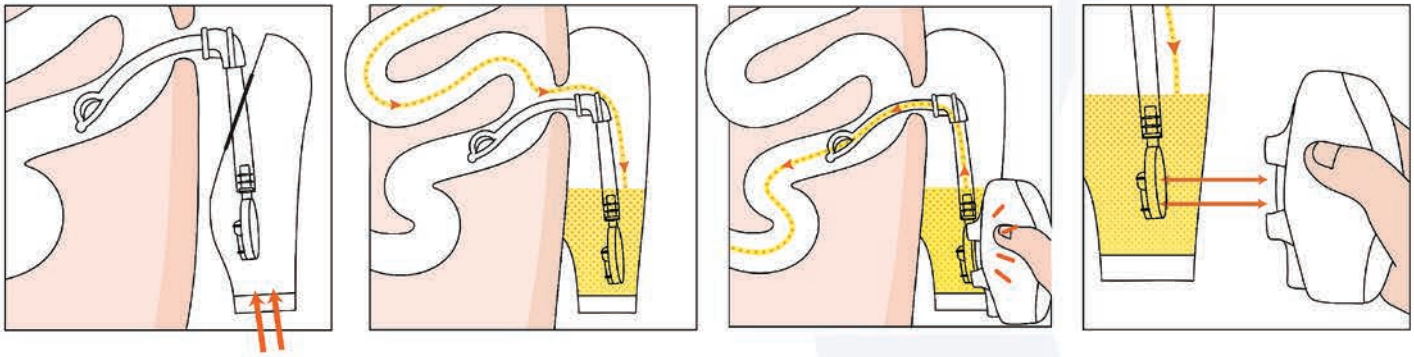

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Possible Treatment Options in Acute Nail Bed Injuries: A Literature Review

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Review Article

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Abstract

The nail is a unique cutaneous structure that plays an important role in hand function, facilitating pinching and increasing the sensitivity of the fingertip. Nail bed injuries are a very common part of hand injuries. In about half of cases, they include distal phalangeal fractures. Injuries that are overlooked or mistreated lead to deformities, which can be dysfunctional or disfiguring. Nail bed injuries are divided into subungual hematoma, nail bed injury with distal phalangeal fractures, and nail avulsion injuries. All these injuries require careful evaluation and adequate treatment. This review article addresses possible treatment options for nail bed injuries.

Introduction

The nail is a unique cutaneous structure found only in primates (1, 2). The function of the nail is to protect the dorsal surface of the distal phalanges and increase the sensitivity of the fingertip (1, 3, 4). It helps in picking up small objects, gives support to the digit, and plays a fundamental cosmetic role (1, 3, 5). Loss of the nail is not only aesthetically disfiguring but also dysfunctional (1, 6).

The nail consists of a nail bed, a nail fold, a nail plate, and surrounding soft structures, which are the *perionychium*, *eponychium*, and *hyponychium* (Figure 1) (1, 3). The nail bed is a smooth, thin, flat tissue with rich blood supply composed of the proximal germinal matrix and distal sterile matrix, and it is the part that the nail adheres to (1, 7, 8). The site where the two matrices meet is located at the level of the *lunula* (3). The nail fold consists of the germinal matrix and *eponychium* (1). More than 90% of ungual keratin production or nail growth is attributed to the germinal matrix, and the sterile matrix contributes to adherence (1, 3).

The fingertip is the most commonly injured part of the hand, in which the nail bed is injured in 15–24% of cases (6, 7, 9). Most

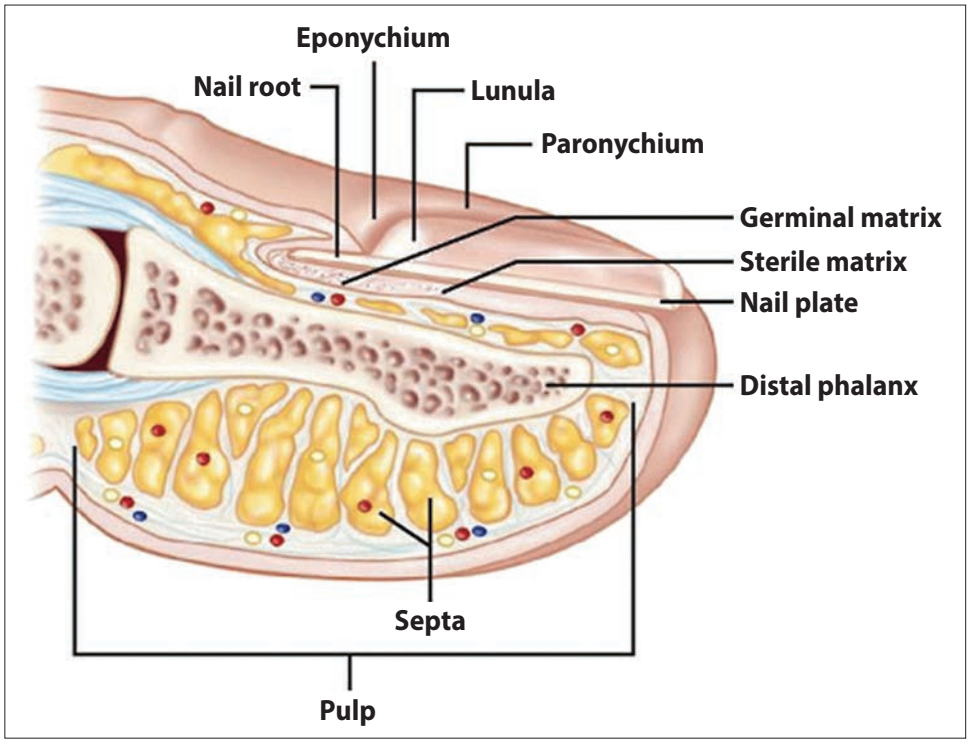

Figure 1. Anatomy of the nail.

fingernail injuries are either crush/blunt or sharp trauma (9). Injuries can be classified as open (lacerations and avulsions of nail matrix, and amputations) or closed (subungual hematomas) (1, 3, 9). In about 50% of cases, they include phalangeal fractures (3). Nail bed injuries are more likely if there is a phalangeal fracture or subungual hematoma with a surface area of more than 50% (1, 3).

Because the nail adheres to the nail bed, any injury to the nail bed heals with scarring, which can lead to non-adherence and nail deformities (1, 6, 8). Scarring on the germinal matrix leads to no nail growth or a split nail, and scarring on the sterile matrix leads to a split nail or detachment of the nail (9).

Despite quite a high number of cases seen in the emergency department, nail bed injuries are often underestimated and easily overlooked. Injuries that are overlooked or not appropriately treated can lead to visual and functional deformities. This article presents possible treatment options for nail bed injuries. We performed electronic searches in PubMed and the Ovid database based on treatment of nail bed injuries. We included review articles, research articles, and case reports.

Treatment Options

Subungual Hematoma

Closed fingernail injuries can result in a subungual hematoma or collection of blood under the nail plate (1). Small subungual hematomas can be left intact, whereas hematomas that involve more than 50% of the nail plate must be evacuated through one or more holes in the nail plate (1–3). To make a hole, one can use a no. 10 surgical blade, hot wire, or 18 G needle (1, 2, 9). In the case of a distal phalangeal fracture and hematoma involving more than 50% of the nail plate, removal of the nail plate and examination of the nail bed using surgical loupes is mandatory (1–3, 9, 10). Primary repair of nail bed injuries leads to good to excellent results in 90% of cases, as shown by Zook et al. (11).

Special attention should be given to chemical burns that involve the *hyponychium* but without noticeable subungual hematoma. Recommendations for such cases are also removal of the nail plate and evaluation of underlying injuries (12). Lacerations of the nail bed should be treated with fine 6-0/7-0 absorbable monofilament suture

(1–3, 9). Strauss et al. used Dermabond to repair nail bed lacerations. Their conclusion was that the repair was faster with regard to suturing, with the same cosmetic and functional results (2).

As for children, Roser and Gellman reported in their study that the results for simple nail trephination for subungual hematoma were equivalent or even superior compared to ablation and nail bed repair (2).

Nail Substitute

After nail bed exploration and washout of contaminated materials, it is best to preserve the nail plate and use it to cover the previously exposed nail bed (1–3, 9). Ogunro reported that normal nail growth may be obtained when the residual nail bed is effectively covered (2, 3).

The old nail can be sutured using a figure-of-eight suture (Figure 2), preferably avoiding the needle going through the nail bed (1–3).

Some surgeons also use simple four-quadrant sutures (2). The new fingernail is expected to grow in about 1–3 months, pushing the old nail off. For better blood drainage and to avoid any complications, a few holes are made in the nail plate (1, 3). Reattaching the nail has several functions: it works as a splint to decrease postoperative pain, supports possible phalangeal fractures, improves tactile sensation during healing, shapes the nail bed fragments, and avoids adhesion between the roof and nail bed. Good insertion of the nail into the proximal nail fold is especially important to prevent adherence between the nail matrix and *eponychium* and subsequent ungual dystrophy (2, 3). If the nail plate is lost or completely damaged, a nail substitute should be used (2, 3). Some surgeons use nonadherent gauze or a polyurethane sponge, but they do not work as a splint (3). Others use a silastic sheet, plastic cut to nail shape, or flexible polypropylene foil shaped like the nail, and Ogunro described prosthetic splints (INRO Surgical Nail) (2, 3).

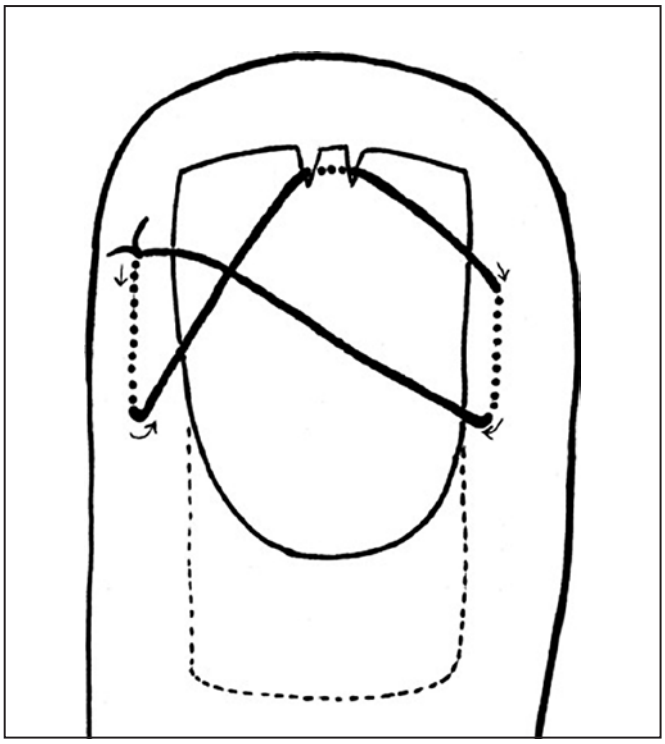

Figure 2. Figure of eight suture.

Distal Phalangeal Fractures

Distal phalanx fracture is seen in approximately 50% of nail bed injuries (2, 3). If the fracture is non-displaced, even with a comminuted fracture, only nail bed repair and nail replacement are necessary (2, 3). Unstable, displaced fractures and fractures of the middle and proximal part of the distal phalanx usually require Kirschner wire fixation (2, 3). Fixation is done with 0.8 mm Kirschner wire in a longitudinal or crossed manner (3).

Nail Avulsion Injuries

Sometimes fingertip injuries present with a nail bed defect (Figure 3). To prevent healing by secondary intention, which leads to chronic deformities, various treatment options for treating such injuries exists (1, 6, 8). These options include split-thickness and full-thickness nonvascularized nail bed grafts, composite grafts from the severed segment, dermal skin grafts, artificial dermis or acellular dermal matrix, rotational nail bed flaps, local/distant flaps, and microvascular free nail transfer (4–8, 10–16).

Nail Bed Grafts

Various authors have described using split-thickness and full-thickness grafts for reconstruction of nail bed defects (11). A treatment algorithm created by Koh et al. divides nail bed defects into

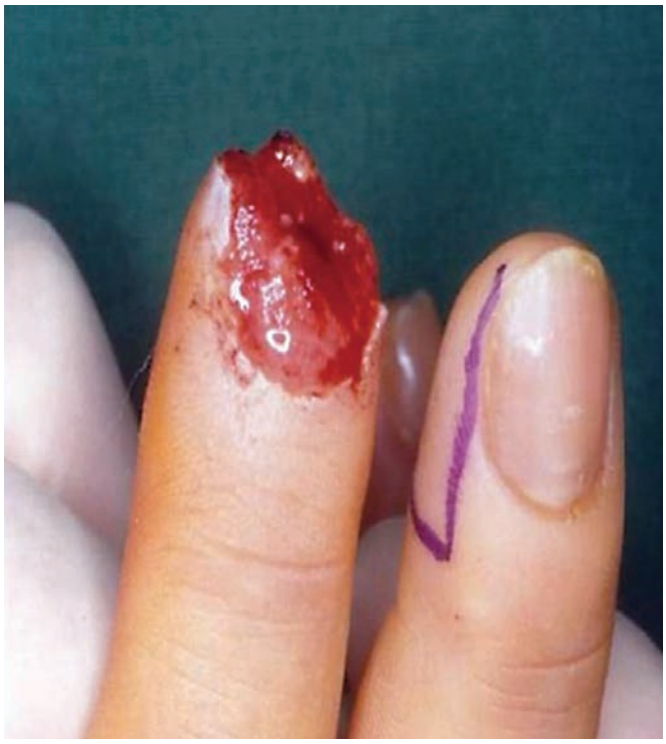

Figure 3. Nail bed defect.

those with and without a severed segment. If there is a severed segment, a composite graft is used. If there is no severed segment and there is no bone exposure, or if it is relatively small, nail bed grafts are used. For larger bone exposure, a nail bed graft is coupled with a flap (10).

A nail bed graft can be taken from the uninjured area of the finger involved, a "bank" finger, or an uninjured finger or the big toe (3). A split-thickness graft has less donor site morbidity compared to full-thickness, but with more common nonadherence of the growing nail (3, 6, 11). It is necessary to use a full-thickness graft when there is a defect of the germinal matrix (3), with 0% chance of success using a split-thickness graft as described by Pessa et al. (11).

The germinal and sterile matrix must be reconstructed separately. When reconstructing the sterile matrix, one should be aware of the longitudinal ridges. It is essential to match the direction of the defect during harvesting and grafting of the defect (10). At all times, the graft must be 1–2 mm larger than the defect (6).

Should reconstruction with a split or full-thickness graft be unsuccessful, a nonvascularized composite nail bed graft (combining sterile and germinal matrices with or without the dorsal roof) can be used instead of technically demanding microvascular transfer (4, 6).

Dermal Skin Flaps

Reconstruction of the nail bed, especially the distal sterile part, can also be performed using a dermal graft in the reverse fashion. Clayburg et al. described this procedure, in which an elliptical portion of full-thickness skin from the palmar side of the wrist crease was excised, defatted, and deepithelialized. The harvested dermal graft is tailored to fit the nail bed defect and is inset in a reversed manner. It is also possible to only raise a thick split-thickness skin flap with a dermatome to reveal the underlying dermis, which is then excised. The greatest advantage of dermal skin flaps is their rich dermal plexus of vessels. Dermal skin flaps are not appropriate for treating germinal matrix defects because of their very low success rate (8).

Dermal Substitute

Acellular dermal matrix or artificial dermis can be used to prevent donor site morbidity in covering nail bed defects (11, 13, 14). Acellular dermal matrix is usually a single layer, composed of a porous matrix of bovine collagen. It is used as an alternative for split-thickness or full-thickness nail bed grafts, functions as a scaffold, and promotes granulation tissue ingrowth. Fiedler et al. reported its use in nail bed avulsions and crush injuries, without inclusion of the germinal matrix, with excellent results (11, 14). Their operation was performed in one stage, first by covering the bone exposure with an autogenous fat graft and then applying an acellular dermal matrix (11).

In their series, Lie et al. applied an acellular dermal matrix for 3 weeks until the defects with bone exposure and germinal matrix damage showed full granulation with subsequent full-thickness skin grafting (14).

Artificial dermis can also originate from pigs or calves and consist of two layers. It can also be applied directly on an exposed phalanx. In his study, Sugamata showed good results with artificial dermis without grafting (13).

Rotational Nail Bed Grafts

In treating larger defects, measuring 5–6 mm, especially if they are full thickness, it is possible to use the well-vascularized nail bed as one or more rotational flaps (3, 12). Such an approach is faster and involves a shorter time until returning to work, which is more favorable for occupational workers (12).

Local and Distant Flaps

Despite somewhat inferior results compared to microvascular toenail transfer, flaps can be applied for reconstruction of large full-thickness nail bed defects with an exposed distal phalanx (2, 7, 16).

Nail bed defects can only affect the sterile matrix, and injuries to the germinal matrix should not be fixed using local flap coverage (16).

There are various types of local flaps described in the literature for covering such defects: Atasoy and Kutler V-Y flaps, adipofascial flaps, cross-finger fascial flaps, thenar fascial flaps, and reverse digital artery flaps (2, 7, 14, 16). Flaps were usually covered with split-thickness toenail bed grafts, using a single-stage or two-stage procedure (7, 16).

Sabapathy et al. reconstructed fingertip amputations with volar advancement flaps and free nail bed transfer. Hwang et al. used volar V-Y advancement flaps for transverse or dorsal injuries of the fingertip and abdominal flaps for volar oblique injuries (16). Abdominal flaps are also used for multiple finger injuries, but they are often too bulky and insensate (2).

Yang et al. used a cross-finger fascial flap combined with split-thickness nail bed graft from the big toe to reconstruct large defects of the sterile matrix. He described several advantages: excellent blood supply for the nail bed graft, preserved length of the digit, a suitable and easy surgical technique, preservation of the nail, and minimal trauma to the donor site. The only contraindications are germinal matrix injury and multiple fingertip injuries (16).

In contrast, Lee et al. used a two-stage procedure, first covering the exposed distal phalanx with a thenar fascial flap and then using flap division with split-thickness nail bed grafting from the big toe. A thenar fascial flap can only be used for the index, middle, or ring finger and, like other flaps, it should not be considered for germinal matrix injury (7).

Microvascular Transfer

Traumatic amputations of the fingertip including distal phalangeal bone loss can be reconstructed using various free toe transfers if re-implantations are not possible (5, 15). Partial toe transfer is performed when there is distal phalangeal bone loss; it includes a trimmed big toe tip and second toe tip transfer, reduced second toe transfer, second toe distal phalanx transfer, onychocutaneous flap, and osteo-onychocutaneous flap (15). When there is no phalangeal bone loss, one can perform a free nail flap with or without hemipulp skin and nail flap with hemipulp skin and plantar skin (5).

The donor site used to be covered with split-thickness graft or a cross toe flap from the undersurface of the adjacent toe, but today we use artificial dermis and secondary skin grafting (5).

Nail Deformities

When nail bed injuries are not appropriately treated, chronic deformities occur (1, 6, 8). The most frequent are pincer nail, split nail, hook nail, ridged nail, and *onycholysis* (1).

Treatment of chronic changes remains controversial. There are number of different procedures available: excision of matrix scar, free nail bed grafting, free nail transfer, nail ablation and split-thickness skin graft, and nail prosthesis (8).

Lemperle et al. discovered that an *en bloc* crescent-shaped excision of scarred nail bed, down to the periosteum, stimulates the matrix cells of the proximal nail bed to grow faster (18). This technique restores the visible length of the nail in a similar manner as Bakhach's eponychial flap (17, 18).

Discussion

To summarize which treatment option provides the best result, it is necessary to consider functional and aesthetic results, and the time of return to work.

Lille et al. stated that the most reliable results are achieved with free-vascularized nail transfer of the whole toenail (4). Although this method is very technically demanding and time-consuming,

it produces minimal postoperative scarring and minimal sacrifice of the donor site (4, 15). The success of nail bed replacement is inconsistent when tissues other than those of the nail bed are used, as stated by Hsieh et al. (6).

Many patients with nail bed injuries are occupational workers, for whom the time of return to work is the most important factor. For them, rotational nail bed flaps for nail bed reconstruction are the optimal treatment option (2, 12).

Sung et al. stated that there were no significant differences in outcome depending on the reconstruction method, except for when the germinal matrix was damaged. Nail bed grafting of germinal matrix defects has a tendency to scar and causes problems with nail bed production (10).

On the other hand, a two-stage procedure for defects of the whole nail bed and germinal matrix loss with artificial dermal matrix coverage and skin grafting provided satisfactory aesthetic outcome with minimal donor site morbidity. The problem is a longer hospital stay and that it was only performed for defects following oncological excisions (14).

Regardless of the techniques described above, the optimal treatment option remains acute nail bed exploration and accurate approximation (4).

Conclusion

Nail bed injuries are seen quite often. Injuries that are overlooked or mistreated can lead to chronic deformities that are not only disfiguring but also dysfunctional. Accurate and timely diagnosis, and appropriate repair are critical for any nail bed injury. One cannot overlook the fact that injury to the nail is not only injury to the nail bed, but to the entire fingertip complex, including the skin, soft tissue, and distal phalanx.

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Comorbidity and Postoperative Complications as Negative Predicting Factors for In-Hospital Mortality in Surgical Patients with COVID-19

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Key words

COVID-19, comorbidity, postoperative complications, mortality, predictive factors

Research Article

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Abstract

Background. This study determines the correlation between patients' comorbidities and postoperative complications as prognostic factors and in-hospital mortality.

Methods. Thirty-seven patients with COVID-19 that were operated on between November 3rd, 2020 and January 10th, 2021 were included in our study. We analyzed the correlations between their comorbidities, postoperative complications, and status at discharge.

Results. A significant association between postoperative complications and status at discharge was observed (p = 0.011), as well as a significant correlation between the comorbidity group and status at discharge (p = 0.027). A cutoff value of age 64 years predicts mortality with a sensitivity of 100% and specificity of 61.8%, and it is significantly associated with perioperative mortality (p = 0.039). Postoperative morbidity was 22.2%, with pneumonia being the most prevalent cause of morbidity, and it was observed in 5.6%. Only 16.3% of patients needed ICU admission. Cumulative mortality was 8%. The median hospital stay was 10 days, and the readmission rate was 11.1%. Multivariate binary logistics analysis identified no significant predictor for mortality for the sample analyzed.

Conclusions. Multiple comorbidities and age over 64 were significant risk factors for postoperative mortality. These factors might be valuable when selecting patients for elective operation during the COVID-19 pandemic.

Introduction

Many studies have confirmed that SARS-CoV2-2 infection significantly increases the risk of perioperative morbidity and mortality in surgical patients (1, 2). The risk of complication is greater in symptomatic patients, with postoperative mortality rates as high as 30% in emergency settings (2–6). Moreover, patients with COVID-19 admitted for elective surgery were reported to have a 12-fold increased risk of mortality compared to patients without COVID-19 (7, 8).

The most important factors associated with mortality were male sex, age 70 years or older, ASA grades 3–5, emergency, major surgery, and malignant diagnosis (7, 8). In addition, numerous chronic diseases that are common in today's geriatric population—such as hypertension, diabetes with chronic complications, hemiplegia, renal disease, malignancy, and liver disease—have been found to be associated with increased mortality in concomitant COVID-19 infection (9). These patients have been reported to be prone to general complications such as pulmonary and thrombotic complications, cardiac arrest, sepsis, and acute renal failure (3, 7, 10). In addition, surgical complications are also increased in these patients, with surgical site infection cited as the most common complication associated with COVID-19 (7, 10). All these complications have led to increased intensive care unit admission rates compared to patients without COVID-19, reaching up to 43% according to various reports, all leading to increased mortality (6, 7, 11, 12).

Because surgery for patients is indispensable despite the COVID-19 pandemic, it is imperative that risk factors for perioperative complications and mortality be identified. With better knowledge of such factors, patients at risk could be identified and preventive measures could be undertaken to make surgery safer. Unfortunately, due to heterogenous patient groups included in the studies and a small number of patients, exact cutoff points for stratifying patients' risk could not be determined. Therefore, we performed a study that analyzed the results of emergency and elective surgery in patients with COVID-19 to determine the risk factors for morbidity and mortality and to better select patients that should not be operated on during acute infection due to increased risk of mortality. Such data and their evaluation can shed light on the surgical risk profiles of patients with and without COVID-19 infection, which can be of great help during preoperative decision-making and patient optimization to improve surgical outcomes.

Methods

Patients

Thirty-seven patients infected with COVID-19 and admitted for elective and emergency surgery at the Maribor University Medical Center in Slovenia between November 3rd, 2020 and January 10th, 2021 were included in our study.

The demographic baseline characteristics of the patients was obtained and stored in our database at admission. Patients' perioperative results were analyzed. Our primary objective was to describe postoperative in-hospital and 30-day post-discharge mortality and morbidity rates in patient groups positive and negative for COVID-19 at the Department of Abdominal and General Surgery at the Maribor University Medical Center. Our secondary endpoints were readmission rates, hospital length of stay, and intensive care unit admission rates.

The data were blinded to prevent any sensitive data from being disclosed. All patients provided their informed consent. The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the local ethics committee.

Operations

All elective and emergency operations were performed according to current surgical standards. COVID-19 infection did not influence our decision in any way to perform standard operations according to currently accepted guidelines. All surgery was performed in accordance with strict protocols for individuals with highly infective disease. This means that operations were performed in a specially designated operating theatre under quarantine conditions. Only personnel with protective garments were allowed to be in direct contact with the patients and in the operating theatre. After the operation, the patients were

transferred either to a general quarantine ward or to a quarantine intensive care unit depending on the general state of the patient and the extent of the operation. Patients were kept under quarantine until at least the 10th postoperative day, ensuring a negative PCR test.

Study Endpoints

Our primary endpoint was postoperative in-hospital mortality in selected patients. Our secondary endpoints were readmission rates, hospital length of stay, and intensive care unit admission rates.

Statistical Analysis

Continuous data were presented as mean ± SD or median (IQR) where appropriate. Discrete variables were presented as absolute values (%). The distribution of the data was analyzed with the Kolmogorov–Smirnov test and Shapiro–Wilk test. Continuous variables were compared with Student's *t*-test or the Mann–Whitney *U* test where appropriate, and the discrete variables were compared with the χ^2 test. The correlation between demographic data, type of surgery, extent of surgery, diagnosis, comorbidities, and morbidity or mortality was determined with Pearson's correlations. Factors with a *p*-value less than 0.4 were included in the multivariate analysis. Significant predictors for perioperative morbidity and mortality were accessed with logistic regression analysis. Significant cutoffs for prognostic factors were determined with ROC analysis. For the level of significance, a *p*-value less than 0.05 was selected. All analysis was performed with SPSS v 25 for windows (IBM SPSS Statistics for Windows, version 25.0 Armonk, NY: IBM Corp).

Results

Patients

Among 292 patients admitted between 2019 and 2020, 61 were infected with COVID-19. Among these, 37 were operated on and included in our study. Three patients died during their treatment. The clinicopathological characteristics of the patients included are presented in Table 1. Among the patients, 48.6% had an associated comorbidity. The most prevalent associated comorbidity was cardiovascular disease (5.4%), and 35.1% of patients had multiple associated chronic diseases. Most of the patients underwent elective surgery and were most commonly operated on for malignant disease. Major surgery was performed in 54.1%. Postoperative morbidity was 22.2%. Pneumonia was the most prevalent cause of morbidity and was observed in 5.6%. Intensive care unit admission was necessary in 16.2%. The median hospital stay was 10 days, and the readmission rate was 11.1%. The cumulative mortality was 8%.

Comparison of the Characteristics of Patients with COVID-19 with and without Mortality

The characteristics of patients with COVID-19 with and without mortality are presented in Table 1. There were no significant differences in age, sex, hospital stay, costs, type of operation, or comorbidities between groups. Patients that died during their treatment had significantly more postoperative complications.

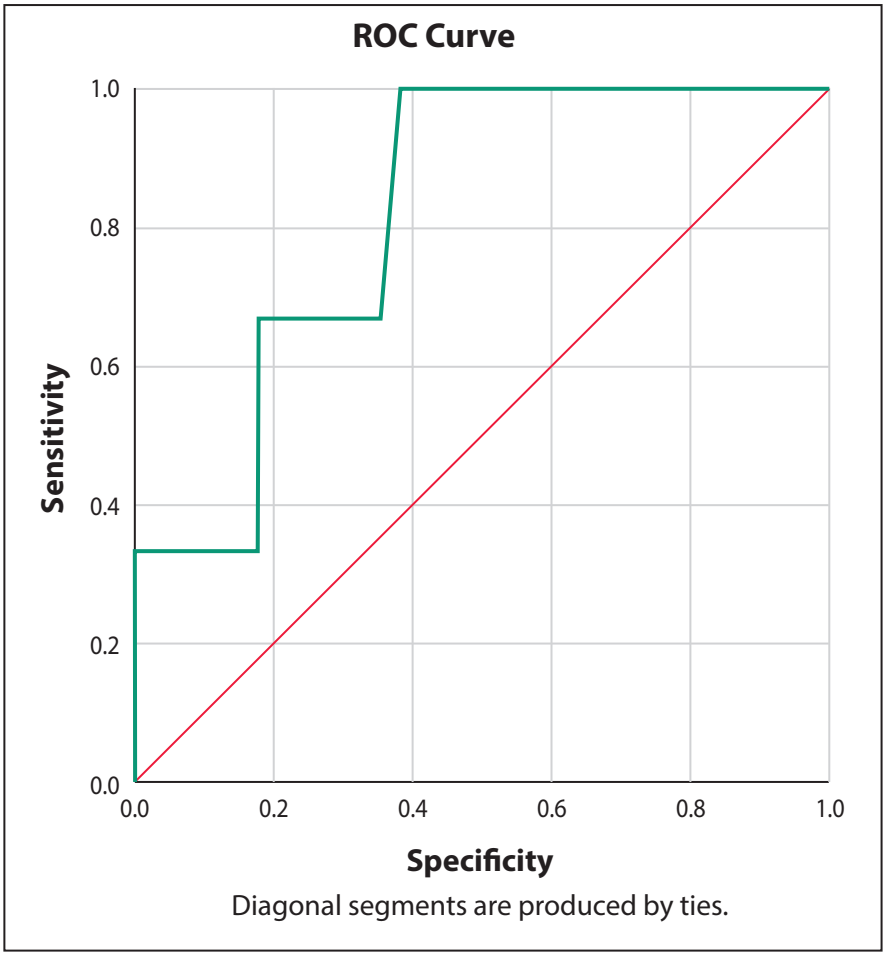
Pearson Correlation between Clinicopathological Characteristics and Mortality

The correlation between demographic data (sex, age), type of surgery (elective vs. urgent), extent of surgery (minor vs. major), diagnosis (diagnosis group), comorbidities (comorbidity group), and morbidity (postoperative complications) or mortality (status at discharge: dead or alive) was determined with Pearson correlations. A significant correlation between comorbidity group and status at discharge was observed ($p = 0.027$).

Table 1. Demographic and clinicopathological characteristics of patients.

Variables		Patients with COVID-19			*p*
		Total (n = 37)	Alive at discharge (n = 34)	Dead at discharge (n = 3)	
Sex, n (%)	Male	22 (59.5)	21 (61.8)	1 (33.3)	0.336
	Female	15 (40.5)	13 (38.2)	2 (66.7)	
Comorbidity group, n (%)	None	19 (51.4)	19 (55.9)	0 (0)	0.304
	Asthma	1 (2.7)	1 (2.9)	0 (0)	
	Cardiovascular disease	2 (5.4)	2 (5.9)	0 (0)	
	Previous COVID-19 pneumonia	1 (2.7)	1 (2.9)	0 (0)	
	Polymorbid	13 (35.1)	10 (29.4)	3 (100)	
	Other	1 (2.7)	1 (2.9)	0 (0)	
Emergency or elective surgery, n (%)	Elective	23 (63.9)	12 (36.4)	1 (33.3)	0.917
	Urgent	13 (36.1)	21 (63.6)	2 (66.7)	
Grouped main diagnosis, n (%)	Neoplasm	13 (35.1)	12 (35.3)	1 (33.3)	0.952
	Gastroenterocolitis	2 (5.4)	2 (5.9)	0 (0)	
	Acute abdomen	16 (43.2)	14 (41.2)	2 (66.7)	
	Pancreatitis	2 (5.4)	2 (5.9)	0 (0)	
	Fasciitis or bedsore	2 (5.4)	2 (5.9)	0 (0)	
	Other	2 (5.4)	2 (5.9)	0 (0)	
Cancer patient, n (%)	Yes	13 (35.1)	12 (35.3)	1 (33.3)	0.946
	No	24 (64.9)	22 (64.7)	2 (66.7)	
Extent of procedure, n (%)	Major	20 (54.1)	18 (52.9)	2 (66.7)	0.647
	Minor	17 (45.9)	16 (47.1)	1 (33.3)	
Postoperative complications, n (%)	None	28 (77.8)	28 (82.4)	0 (0)	0.011
	Sepsis	1 (2.8)	1 (2.9)	0 (0)	
	Pneumonia	2 (5.6)	1 (2.9)	1 (50)	
	Other	5 (13.9)	4 (11.8)	1 (50)	
ICU admission, n (%)	Yes	6 (16.2)	5 (14.7)	1 (33.3)	0.401
	No	31 (83.8)	29 (85.3)	2 (66.7)	
Readmission, n (%)	Yes	4 (11.1)	4 (11.8)	0 (0)	0.607
	No	32 (88.9)	30 (88.2)	2 (100)	
Age, years		53 ± 21	51 ± 21	72 ± 10	0.104
Hospital stay, days		10 [22–6]	10 [24–6]	4 [/]	0.118
Number of admissions, n		1 (1–1)	1 (1–1)	1 (1–1)	0.666
Costs, €		8,910 (14,533–2,318)	8,910 (14,533–2,318)	Not specified	

Figure 1. ROC curve for age.



The ROC analysis was used to determine the cutoff value for age. The cutoff age of 64 was selected for further analysis because it predicted mortality with a sensitivity of 100% and a specificity of 61.8%. The ROC analysis is presented in Figure 1. The selected cutoff was significantly associated with perioperative mortality (0.039).

Multivariate Analysis

Factors with a *p*-value less than 0.4 were included in the multivariate analysis. The multivariate binary logistics analysis identified no significant predictor of mortality for the sample analyzed.

Discussion

This study observed a significant association between postoperative complications and status at discharge ($p = 0.011$), as well as a significant correlation between comorbidity group and status at discharge ($p = 0.027$). The multivariate binary logistics analysis in our study identified no significant predictor for mortality for the sample analyzed.

It is worth noting that mortality rates were shown to be similar in pandemic and pre-pandemic periods (13). It has been documented that the odds of perioperative complications were significantly higher in patients with COVID-19, occurring in up to 50% of cases (14). Surgical site infection was also more common in those with COVID-19 (7, 10). In our patient group, we observed complications in 22.2%, which is smaller than the percentages reported in some studies (7, 10, 12).

Comorbidities have been shown to add to the mortality of COVID-19 patients (8). Interestingly,

this was not the case in our study. Most of the patients included suffered from multiple accompanying diseases; meanwhile, no correlation was found between comorbidities and mortality in our study.

In addition, despite the relative high proportion of major surgery in our study, only 16.3% of patients needed intensive care unit admission. In comparison, intensive care unit admission rates and the need for mechanical ventilation exceeded 43% in COVID-19 surgical patients (6, 7, 11, 12). It is difficult to explain this difference because we do not have insight into the patient data from other studies. It is therefore possible that in other series more patients had pulmonary-associated diseases or were older. One explanation might be the hospital policy. In our hospital, after general surgical procedures most patients are admitted to the intensive care unit on the surgical ward, and admissions to the intensive care unit are restricted to patients requiring mechanical ventilation.

The median hospital stay was only 10 days in our study, which is the same as in a study published by Haffner et al. (4). This might be explained by the relatively low perioperative complication rate in our cohort.

Only three COVID-19 patients died after surgery. Patients with perioperative morbidity and multiple accompanying diseases were at higher risk of developing complications. Similar observations were observed in various other studies, which reported that patients with COVID-19 had higher mortality (3, 5, 7, 10). Although the multivariate analysis failed to confirm comorbidity and perioperative morbidity as significant predictors for death, we agree that both factors are important prognostic markers for surgical morality in COVID-19 patients. Therefore, whenever possible, patients with multiple accompanying diseases should be rescheduled for elective operation or treated conservatively until the COVID-19 infection resolves.

Univariate analysis identified age as a significant predictor for mortality after surgery in COVID-19 patients. Specifically, age 64 was determined as the cutoff for a poorer prognosis after surgery. Many studies have similarly shown that age is an important predictor for morality in patients after surgery. A study by Fernandez-Martinez et al. (8), as well as a study by COVIDSurg Collaborative (14), showed that age 70 years or older is an independent risk factor for mortality in surgical patients with COVID-19, whereas Rasslan et al. (6) report an age of 75 to 84 as being a predictor for mortality. To the best of our knowledge, this is the first report that an age of 64 was shown to be a negative prognostic marker for morbidity after surgery in COVID-19 patients. Although age was not determined to be a significant predictor in the multivariate analysis, we believe that age should be always one of the most important safety factors when considering surgery in COVID-19 patients.

Our study has some limitations. Due to the small sample size, some correlations that might have become apparent in a larger sample did not attain the level of significance. The severity of COVID-19 infection was not considered when analyzing the patients' data.

In conclusion, our analysis determined that COVID-19 patients with postoperative complications were more likely to die during treatment for a surgical disease. Multiple comorbidities and age over 64 were significant risk factors for postoperative mortality. These factors might be valuable when selecting patients for elective operation during the COVID-19 pandemic.

Institutional Review Board Statement

This study was conducted according to the guidelines of the Declaration of Helsinki. The study protocol was reviewed and approved by the Institutional Ethics Committee at the Maribor University Clinical Center (UKC-MB-KME-74/20).

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Surgical Treatment of Pediatric Hydrocephalus: Lessons Learned at the Ljubljana University Medical Center

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Key words

hydrocephalus, ventriculoperitoneal drainage, ventriculoatrial drainage, ventriculostomy, overdrainage

Research Article

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Abstract

Hydrocephalus is caused by cerebrospinal fluid stagnation in the ventricular system. Its treatment is mostly surgical and involves the insertion of a shunt (in most cases, ventriculoperitoneal or ventriculoatrial drainage) or endoscopic third ventriculostomy. This article describes the surgical treatment of hydrocephalus, the indications for shunting the ventricles, and the techniques used. We also focus on surgical complications. In addition, we briefly describe our treatment results.

Introduction

Hydrocephalus results from an accumulation of cerebrospinal fluid (CSF) within the ventricular system of the brain, and in the vast majority of cases it is related to obstruction of flow. Complex congenital anomalies, neonatal intraventricular hemorrhage, subarachnoid hemorrhage, trauma, tumor, and infection can all cause hydrocephalus. It is associated with developmental delay, neurological deficits, and cognitive disorders. Treatment must be individualized, considering the hydrocephalus severity and etiology and the child's general condition (1, 2).

Children with clinically and radiologically stable and compensated hydrocephalus can be followed for worsening of the condition by regular follow-up visits. The most common treatment of hydrocephalus is the implantation of a ventriculoperitoneal shunt. Another successful but controversial technique is an endoscopic third ventriculostomy. All patients with implanted shunts must be followed through their lifetime because shunt independence is an elusive goal for most of them. Every intervention caries a risk. There is also the risk of deterioration due no intervention. Our goal is to briefly summarize the treatment of hydrocephalus in the pediatric population and share our results and insights on this topic.

Theoretical Aspects of Hydrocephalus and Its Treatment

Indications for Shunting

The most common etiologies of hydrocephalus treated with a shunt are intraventricular hemorrhage, myelomeningocele, tumor, aqueductal stenosis, infection, and head injury. Ventriculomegaly can be diagnosed by ultrasound, MRI, and CT. The differentiation between cerebral atrophy and hydrocephaly is possible by recognition of symptoms (irritability, headache, vomiting, lethargy, and seizure) and signs (bulging fontanelle, increasing head circumference, developmental delay, sunset sign, and papilledema) of raised intracranial pressure (ICP). In children with less pronounced symptoms and signs or unconvincing imaging studies, the decision to implant a shunt is less clear cut. Children with compensated hydrocephalus, in a clinically and radiologically stable situation, may simply be monitored by regularly scheduled follow-up visits. They require frequent imaging and psychological testing to allow for early detection of disease advancement. If imaging shows more than mild hydrocephalus, the patient should have a shunt implanted. The threshold for action is lower in children that are 3 years old or younger.

The best timing for placing a shunt remains a subject of debate because the link between ventriculomegaly and cognitive function is not clear. A general rule (3) is that the thickness of brain tissue at the level of the foramen of Monro must be at least 35 mm. This thickness must be reached before the age of 5 months. If it falls below 10 mm, the chances of recovery, even with a shunt, are slim.

There are disease-specific considerations that have to be taken into account when dealing with the decision to implant a shunt in a child. In cases of posttraumatic ventriculomegaly, a shunt is only rarely indicated, and great care must be taken to differentiate atrophy from true hydrocephalus. Of the children affected by a posterior fossa tumor, only a few require a definitive shunt because the tumor removal and an eventual endoscopic third ventriculostomy are the most appropriate treatments for this hydrocephalus (4). On the other hand, children with an open neural tube defect will most probably require a shunt.

When treating hydrocephalus with a shunt, the goals are: 1) to decrease the ICP, 2) to protect the brain from deterioration, 3) to maintain all CSF compartments open and communicating, and 4) to minimize the need for further interventions through the patient's life.

Pathophysiology

In erect position, the CSF dynamics are subject to hydraulic forces. Venous blood drains into the jugular veins and CSF drains into the spinal subarachnoid space. The CSF pathway and the dural venous sinuses move together so that the absolute pressure differential between the two compartments remains the same regardless of the body's position. In this scenario, the ICP is 5 to 15 mmHg in recumbent position, and -5 to +5 in erect position. The major challenge in treating hydrocephalus is to recreate these natural dynamic conditions of CSF physiology (1).

Hydrodynamic Principles of Shunts

The most common surgical technique in the treatment of hydrocephalus remains the insertion of a venticuloperitoneal shunt (VPS). This device is composed of a ventricular catheter, a valve, and a distal catheter. The valves can be grouped into four categories, based on their hydrodynamic characteristics: 1) differential pressure valves, which are most commonly used, 2) siphon-resisting valves, 3) flow-regulated valves, and 4) adjustable valves. The peritoneum is the preferred receptacle of the shunt: it is easy to access and has an excellent absorptive capacity. Putting the shunt into the peritoneum creates hydrodynamic problems. When the patient lies in a supine position, the flow through the shunt is controlled by the differential pressure valve, but in an erect position the differential pressure across the valve is overwhelmed by the hydrostatic forces created by the distance of the tube from the ventricles to the peritoneum. Flow of CSF continues until the ventricular walls collapse or until ICP reaches extremely negative values. Therefore, ICP in erect position in patients with a shunt is often -25 or -35 mmHg. Extremely negative ICP can cause severe postural headaches,

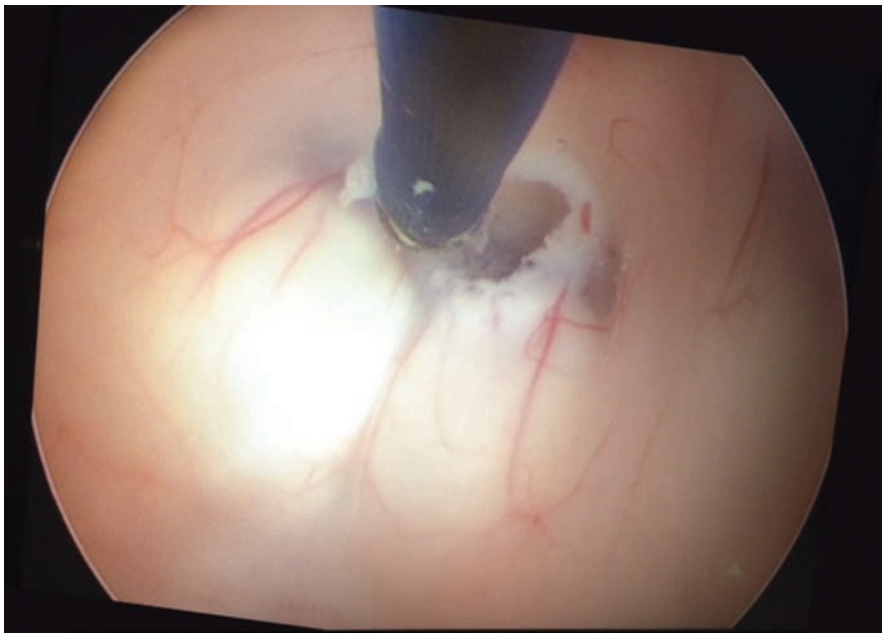

Figure 1. Endoscopic third ventriculostomy.

ventricular collapse, and potentially dangerous subdural hematomas. Many devices have been developed to prevent these problems: antisiphon devices, flow-regulated valves, and gravitycompensating devices (5). Fortunately, the vast majority of patients tolerate these negative pressures very well and most patients worldwide are still treated with inexpensive differential pressure devices. The shunt design trial (6) found that there is no reason to choose one shunt over another because there is no difference in outcome regardless of the type of valve used. The best advice for a surgeon is to become familiar with one system and use it consistently.

Treatment of Hydrocephalus with Endoscopy

Endoscopic third ventriculostomy (ETV) is an alternative treatment for hydrocephalus. The surgical goal is to create an opening in the floor of the third ventricle between the infundibular recess and the mammillary bodies. The procedure can be seen in Figure 1. Not all patients with hydrocephalus are candidates for ETV, and the indications are sometimes still controversial (4). However, several patients that would previously require a shunt are nowadays successfully treated with ETV. As for any other procedure, ETV comes with its own risks and complications: early and late occlusions of the opening and even sudden postoperative deterioration that can lead to death (7).

Follow-up of Shunted Patients

Once children have shunts implanted, the common belief is that they will need it for their entire lives. Shunt independence is a reasonable goal, but not all patients can eventually live without it. Children with communicating hydrocephalus, particularly those with hydrocephalus related to brain tumors and with posthemorrhagic hydrocephalus, had a 50% chance of becoming shunt independent (2). This was not true in the group of children with hydrocephalus related to a Chiari II malformation.

All children with a shunt must be followed throughout their entire lives. A CT or MRI scan should be taken 3–6 months after the implantation because a study at this time has proven to be an adequate baseline for subsequent follow-ups (8). Figure 2 shows good positioning of the proximal catheter on a CT scan. Small children can be monitored with ultrasound until their fontanels close.

In the case of a shunt malfunction, the device needs a revision. Shunt revision is the most common procedure in pediatric neurosurgery. Usually

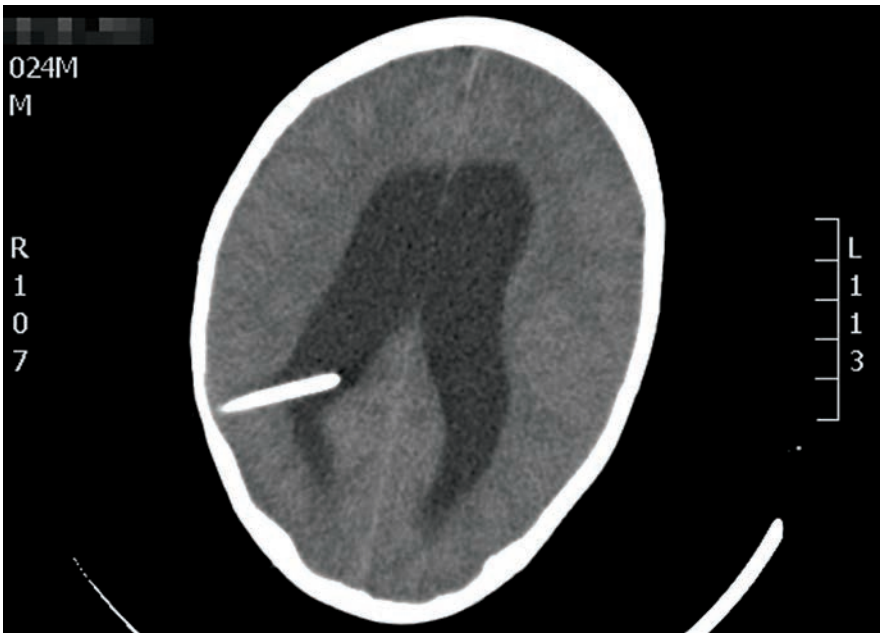

Figure 2. CT scan showing good positioning of the proximal catheter.

the shunt components are separated, starting with separation of the distal catheter from the valve. When the site of obstruction is found, the malfunctioning part needs to be replaced.

Risks of Shunting

The risks of shunting, particularly in children, must not be underestimated. They depend on many factors, age being one of the most important. The younger the patient, the higher the risk. The second factor related to the risk of shunting is the pathophysiology of the condition responsible for the hydrocephalus. A patient with a Chiari II malformation has a 1% risk of death per year, which is higher than in hydrocephalus from other causes. The third risk factor is the complexity of the hydrocephalus itself: if more than one ventricular catheter is implanted, the risk of malfunction or infection is higher.

Another rare but serious complication of shunts is overdrainage. This can produce extra-axial fluid collections (hygroma or subdural hematoma) when occurring in the early postoperative period. The treatment of this condition consists of changing the valve or setting the valve to a higher value.

The second situation, which can result from chronic overdrainage, is known as slit ventricle syndrome. The symptoms of this condition are like those of a shunt malfunction. Most commonly, children complain of intermittent headaches, nausea, and vomiting, which are often related to posture: an improvement is often reported in a supine position. It is a rare, late shunt-related complication that affects 0–6% of all patients. It occurs at least 6 years after shunt implantation. The overdrainage is because of the siphoning effect of the water column in the distal catheter, which causes slit ventricles. The brain fills the intracranial space and the ability to compensate for transient changes in intracranial volume is impaired. Small ventricles that have poor compensatory capacity cause intermittent catheter obstruction, which produce symptoms of elevated ICP even without major changes in ventricular size. Medical treatment with antimigraine drugs is often effective. In the case of a failure to resolve the symptoms, there are several possible surgical techniques: changing or upgrading the valve, adding an antisiphon device, and cranial expansion.

The third condition that results from overdrainage is known as loculation. In this situation, the shunt drains only one lateral ventricle, which results in functional obstruction of the foramen of Monro and a dilated contralateral ventricle. A good strategy for these children is to perform an endoscopic septostomy. A similar condition is seen in the isolated fourth ventricle, a condition that can result in irritability, headaches, cardiorespiratory arrest, and death. The endoscopic insertion of a stent through the aqueduct is the best management of this condition.

A child that grows up with a shunt will remain shunt-dependent throughout his whole life, also bearing the associated risks. In this sense, the transition from pediatric to adult neurosurgical care is also of particular importance because a patient that has had a shunt implanted in childhood behaves differently from someone that received a shunt as an adult. In the case of shunt malfunction, ventricles always enlarge in this second case, but almost never in the first one.

Patients, Methods, and Results

We retrospectively reviewed our series of 64 children treated for hydrocephalus from January 2016 to August 2020 at the Pediatric Neurosurgery Unit of the Ljubljana University Medical Center. The etiologies of hydrocephalus were as follows: neonatal intraventricular hemorrhage in 29/64 (45.3%) cases, idiopathic in 7/64 (10.9%) cases, pineal region tumor in 6/64 (9.3%) cases, supratentorial tumor, myelomeningocele, and posterior fossa tumor in 5/64 (7.8%) cases, subdural hematoma and Blake's pouch in 2/64 (3.1%) cases, and infection, porencephaly, and aqueductal stenosis in 1/64 (1.5%) case. The age of the patients in this cohort ranged from 0 to 15 years.

An ETV was performed in 29/64 (45.3%) cases to avoid the insertion of a shunt. This procedure was successful in 13 cases, corresponding to a success rate of 44.8%. A revision of the ETV was performed in 3/29 (10.3%) cases and was successful in one case. The etiology of the 13 cases that were successfully treated with ETV was related to a tumor in 7/13 (53.8%) cases, Blake's pouch in two (15.3%) cases, and myelomeningocele in one (7.6%) case. In addition, two (15.3%) cases of idiopathic hydrocephalus were successfully treated with ETV.

A VPS was implanted in 48/64 (75%) children in our series. The median age at insertion was 23 months. We used a flow-regulated valve in 25/48 (52%) cases and a differential pressure valve in 20/48 (41.6%) cases. An adjustable valve was used in 3/48 (6.2%) cases.

In our series, 19/48 (39.5%) children presented with one shunt malfunction, and a second shunt malfunction occurred in 7/48 (14.5%) children. Three episodes of malfunction occurred in 5/48 (10.4%) children, and four and five episodes in 2/48 (4.1%) children. The median time from previous shunt surgery to shunt malfunction was 3.7 months (min. 4 days – max. 32 months). Overall, 35 episodes of shunt malfunction occurred. The causes were as follows: a proximal catheter occlusion in 15/35 (42.8%) cases, a valve occlusion in 7/35 (20%) cases, and a distal catheter occlusion in 1/35 (2.8%) case. Other causes of shunt malfunctions were hypodrainage in 4/35 (11.4%) cases, caudal migration of the entire shunt system in 3/35 (8.5%) cases, proximal catheter disconnection in 3/35 (8.5%) cases, and proximal catheter malposition in 1/35 (2.8%) case. All shunt malfunctions were surgically revised. During surgery, the obstructed part or the entire shunt was replaced. In the four cases of hypodrainage, a flow-regulated valve was replaced with a differential pressure valve or an adjustable valve.

We recorded 12 shunt infections in 10 children. The median time from the last surgical procedure to infection was 32 days (min. 3 days – max. 4.4 months). The median age at infection was 4.3 months (min. 19 days – max. 10.3 months). Two children had two separate infections, at a distance of 8 and 11 months. Shunt infection was treated in all cases with removal of the shunt. An external ventricular drainage was temporarily inserted in eight cases, and four cases did not need a temporary device. The new VPS was implanted after a median time of 14.2 days (min. 9 – max. 24 days) from the shunt removal. Overall, in our series, we performed 95 shunt implantations and revisions during a median follow up of 27.5 months (min. 3 months – max. 4.6 years). This shows a median rate of 1.9 surgeries for each child. The infection rate was 12.6%.

Discussion

Despite advances in the field and new technologies, complications related to VPS are still a source of disability and morbidity. Between 30 and 40% of shunts fail during the first two years of life (9, 10). Sainte-Rose (11) also reported a higher rate of failure for shunts implanted in the frontal horn compared to those placed in the atrium through a posterior burr-hole. The most common cause of shunt failure is obstruction, particularly of the proximal catheter. Figure

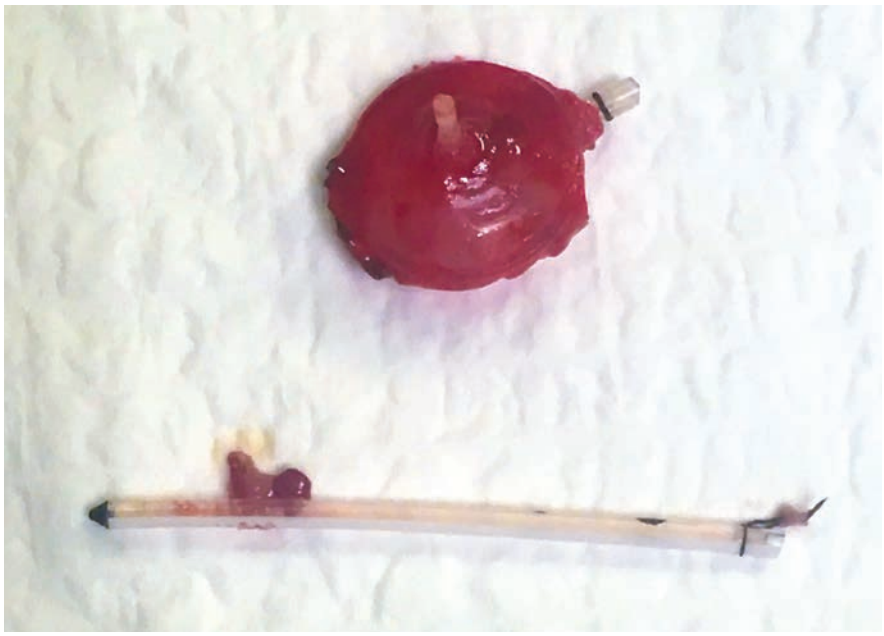

Figure 3. Proximal catheter and valve were removed because of obstruction.

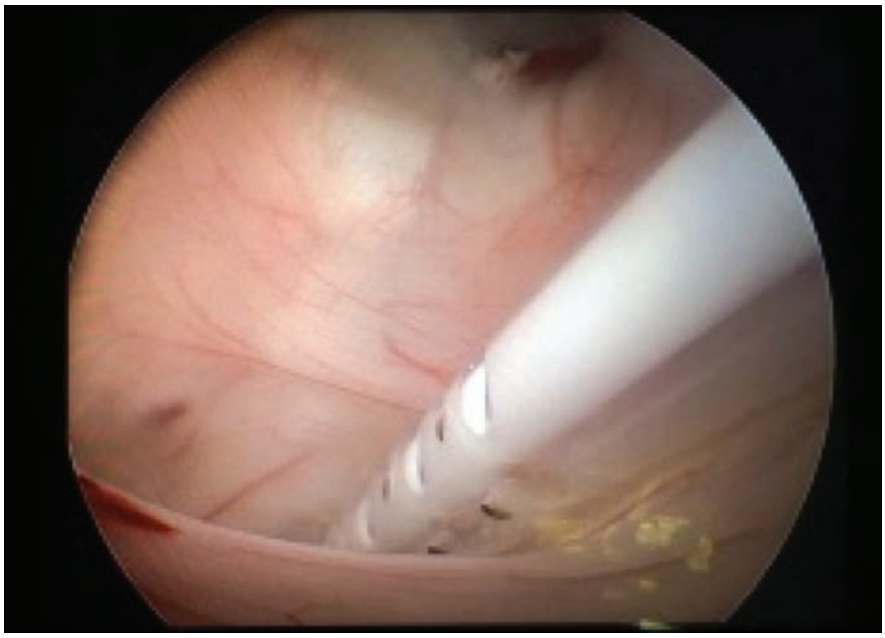

Figure 4. A disconnected proximal catheter.

3 shows a proximal catheter and valve removed because of obstruction. This represented 63% of mechanical complications in one series (9). The shunt system may also disconnect, migrate, or fracture. A disconnected proximal catheter can be seen in Figure 4.

In the shunt trial (6), the infection rate was 8.1%, with the first months of life particularly susceptible to shunt infections. We believe our number to be quite reasonable and probably simply the reflection of a smaller sample size compared to the 344 patients in the trial.

Conclusions

Treatment of hydrocephalus is complex and must always be individualized based on the underlying pathology and the child's characteristics. Whenever possible, endoscopic treatment is preferable because it avoids all risks and complications related to the insertion of a shunt. Despite this, shunts are still the most frequent method of treating pediatric hydrocephalus. Children with implanted shunts need to be followed up throughout their entire life.

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Enterocutaneous Fistula Secondary to Mesh Extrusion After Laparoscopic Intraperitoneal Onlay Mesh Repair (IPOM) for Incisional Hernia in a PolymorbidPatient: A Case Report

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Key words

incisional hernia, IPOM, laparoscopy, enterocutaneous fistula, hernia repair

Case Report

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Abstract

Incisional hernia repair is a frequently performed surgical procedure. Laparoscopic intraperitoneal onlay mesh is one of the established surgical techniques for incisional hernia management. However, precise preoperative evaluation and potential benefits of different surgical techniques should be considered prior to every surgery. We present the case of a 65-year-old polymorbid male patient that underwent laparoscopic intraperitoneal onlay mesh repair for a large ventral incisional hernia 15 months after open segmental small bowel resection due to segmental necrosis. Nonabsorbable prosthetic mesh was fixed using titanium tacks. Two months after hernioplasty, the mesh and tacks started to bulge and extrude through the skin, leading to chronic wound infection and formation of an enterocutaneous fistula. Because of worsening of accompanying diseases, the patient was not capable of undergoing any corrective surgery. He has had multiple checkups and supportive hospitalizations since the initial procedure six years ago.

Introduction

Incisional hernia is a common complication after laparotomy, with its incidence ranging from 2 to 20% depending on the location and size of the incision. There are multiple known risk factors that contribute to the development of hernia. The most common are older age, obesity, chronic obstructive pulmonary disease (COPD), emergency surgery, surgical site infection, and suboptimal fascial closure (1).

Because sutured repair is associated with a higher risk of reoperation for recurrence, mesh reinforcement is mainly used

for incisional hernia repair (2). The laparoscopic intraperitoneal onlay mesh (IPOM) technique has been accepted as a reasonable option for incisional hernia repair since its introduction in the 1990s (3, 4). A prosthetic mesh is placed intraperitoneally beyond the borders of fascial defect and secured with spiral tackers, staples, transfascial sutures, glue, or a combination of these techniques (5).

Laparoscopy with mesh implantation may result in various complications such as infection, adhesions, abscess or seroma formation, fistula development, and chronic pain due to nerve entrapment (2). Furthermore, the type of mesh fixation may also play an important role in the development of complications (6). The cut edges of the mesh and spiral tack anchoring devices have been directly associated with severe adhesions or even bowel perforation, especially where mesh migration had occurred (7, 8). Moreover, the use of mesh fixation devices such as tackers or transfascial sutures could result in severe chronic neuropathic pain due to nerve entrapment (9). The type of mesh fixation also determines the degree of tissue contraction (mesh shrinkage) and potentially affects the incidence of recurrent hernia (6). When used in laparoscopic ventral hernia repair, polypropylene mesh can produce the worst complications, such as an enterocutaneous fistula (10).

We report the case of a polymorbid patient with a large incisional hernia that underwent laparoscopic IPOM hernioplasty and subsequently developed an enterocutaneous fistula with mesh and tack extrusion.

Case Presentation

A 65-year-old male with a history of arterial hypertension, heart failure, hypothyroidism, permanent atrial fibrillation, diabetes mellitus type 2, moderate to severe COPD, and peripheral artery disease was admitted to the emergency department with signs and symptoms of acute intestinal ischemia. Segmental resection of the necrotized small bowel was performed in October 2012. Two weeks later, during the postoperative period, a fascial dehiscence occurred. The fascial defect was closed with interrupted absorbable sutures.

Three months later, an incisional ventral hernia measuring approximately 12 × 7 cm developed. In addition, complete epithelialization of the postoperative wound had still not occurred. Therefore, regular dressings to the chronic wound were applied for the next several months, until the wound healed appropriately and the incisional hernia could finally be managed. In the meantime, the hernia expanded. It reached a size of approximately 16 × 9 cm and started causing discomfort.

Fifteen months after the first surgery, the patient underwent laparoscopic IPOM hernia repair in a regional hospital. Nonabsorbable polypropylene mesh was fixed to the peritoneum using titanium spiral tacks. Immediately after the surgery, the patient was transferred to the intensive care unit due to acute exacerbation of COPD. His respiratory symptoms gradually improved. During the postoperative period, a surgical site infection occurred, which was treated conservatively. The patient was discharged two weeks after the hernia repair.

Soon after discharge, hernia recurrence was evident. Two months after hernioplasty, the polypropylene mesh started to continuously extrude through the skin, leading to a chronic wound infection (Figure 1). In addition, spiral tacks also started to extrude through the skin. Six months later, due to long-lasting chronic wound infection, an enterocutaneous fistula with an average output of 50 to 150 ml/day developed at the site of mesh protrusion (Figure 2).

The patient was evaluated by an anesthesiologist at a tertiary care center, who, due to worsening of the patient's comorbidities, advised against surgical treatment under general anesthesia. Therefore, the patient has had multiple checkups and supportive hospitalizations since the initial procedure six years ago. Acute wound infections, and mesh or tack extrusions were all treated conservatively with antibiotics, local mesh excisions, and tack removals (Figure 3). During this time, the site of fistula formation gradually expanded and led to small bowel prolapse (Figure 4). In addition, during the follow-up period, the patient was started on long-term oxygen therapy due to his COPD and underwent a leg amputation due to worsening of peripheral artery disease.

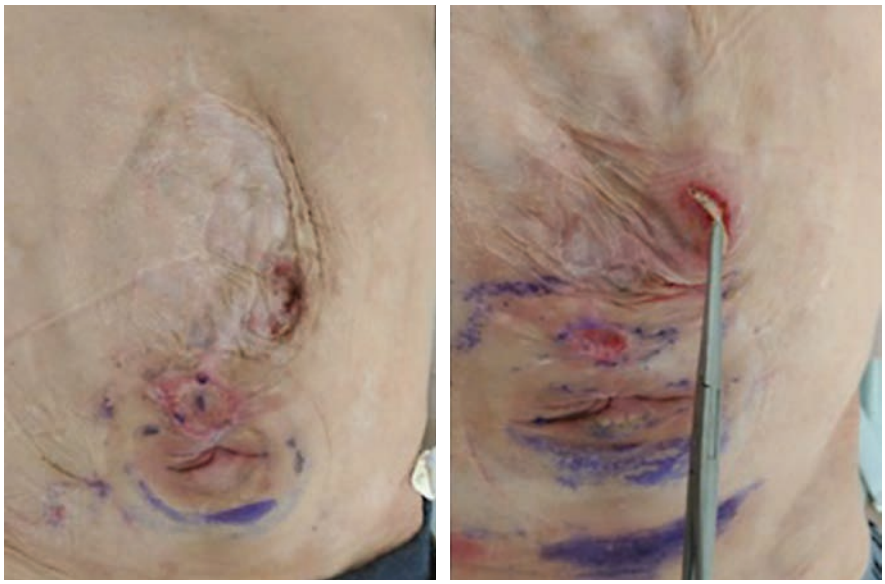


Figure 1. Chronic wound infection due to mesh extrusion.

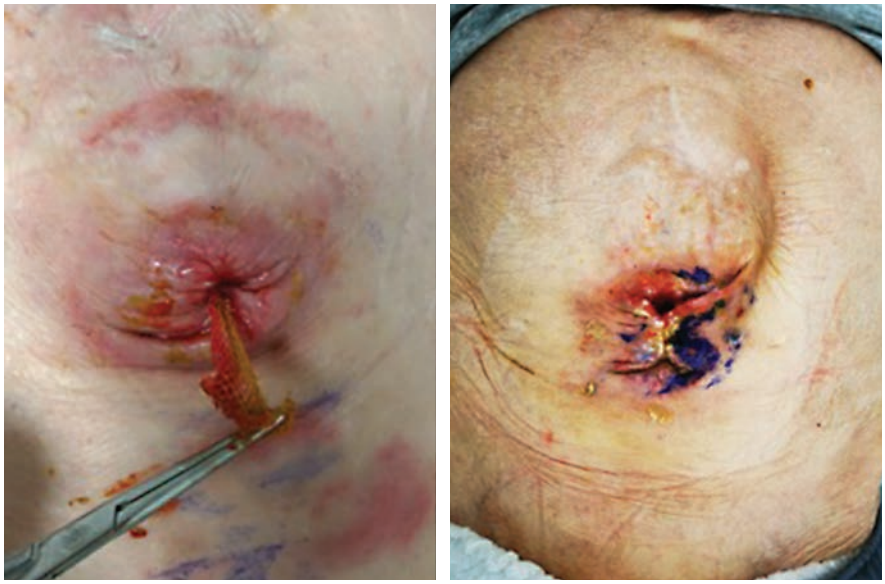


Figure 2. An enterocutaneous fistula at the site of mesh protrusion.

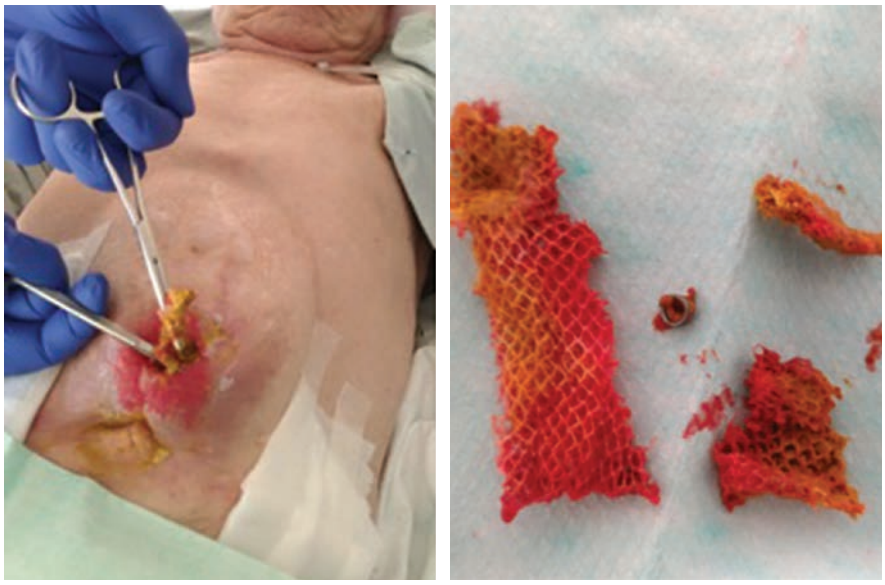
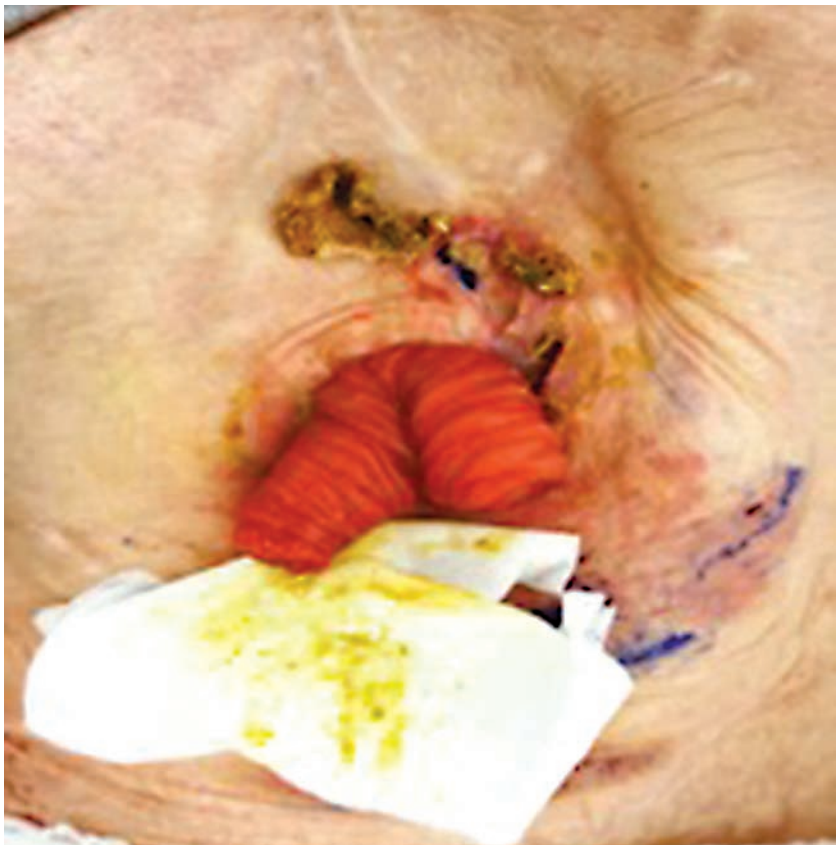


Figure 3. Local mesh excision. Spiral tacks and parts of mesh forming a mesh puzzle.

Figure 4. Small bowel prolapse.



Discussion

Incisional hernia repair is one of the most frequently performed surgical procedures. However, its management still remains heterogenous with little supporting evidence (11).

Numerous studies have compared laparoscopic IPOM versus the open sublay technique for incisional hernia repair. Laparoscopic IPOM hernioplasty leads to a lower incidence of surgical site infections and seroma formation, and to a shorter hospital stay. On the other hand, the cost of the laparoscopic approach is higher, and it is associated with more intraoperative complications, such as bleeding, bowel injuries, and other organ injuries. These tend to be more severe and could greatly compromise a patient's overall quality of life (4). Another study showed that intraperitoneally placed mesh was associated with severe, potentially life-threating complications compared to the modified open sublay approach (12).

As an intra-abdominal foreign body, mesh induces an inflammatory response. This leads to the formation of adhesions between the mesh and the abdominal wall. On the one hand, adhesions are part of the normal healing process because they allow good incorporation of the mesh. On the other hand, they can induce abdominal wall weakening and lead to an increased hernia recurrence rate. Mesh adhesions can also be associated with bowel obstruction, bowel erosion, infertility, fistula, and chronic pain, and they can cause technical difficulties at reoperation. Nevertheless, intra-abdominal adhesions are not specifically related to mesh placement, but they are an inevitable consequence of any kind of surgery (13).

In general, the indication to repair a ventral hernia is for symptom relief and/or prevention of future problems related to the hernia, such as pain, acute incarceration, enlargement, and skin problems (14). Laparoscopic IPOM, specifically, is indicated in patients with recurrent hernias after open repair, in patients with undisturbed abdominal wall function with Swiss cheese defects, and in individuals with small fascial defects with a large primary incision (to cover the entire scar). It is also suitable in patients at high risk for infection (diabetes, obesity, and immune

compromised) because the laparoscopic approach is associated with fewer surgical site infections (15).

The complexity of incisional hernia management goes even further in polymorbid patients with several risk factors for hernia recurrence. It has been shown that incisional hernias are associated with a higher incidence of comorbid diseases compared to other types of abdominal wall hernias (16). Another study evaluated a complex group of patients with clinically relevant comorbidities undergoing incisional hernia repair. They determined that surgical site infection was most common in patients with obesity, diabetes, COPD, and a history of smoking. Laparoscopic repair was reserved for small to medium-sized defects in polymorbid patients (17).

In our case, the patient with several comorbidities underwent laparoscopic IPOM repair for a very large ventral incisional hernia. In addition, the patient had a history of surgical site infection after his first open surgery due to bowel ischemia. He developed an enterocutaneous fistula a few months after hernioplasty, and he has had several supportive hospitalizations since then. However, in this case study we could not elucidate whether the complication occurred specifically due to the laparoscopic procedure, spiral tacks, or nonabsorbable polypropylene mesh.

Management of incisional hernias remains complex, mainly depending on the defect size and the patient's overall condition. Abdominal wall hernias manifest a wide spectrum of disease, and so proper patient selection is paramount because not every patient may be a suitable candidate for laparoscopic intervention. Thus, some patients may be well served by an open approach. There is little supporting evidence and there are few studies addressing management of incisional hernias in patients with several comorbidities and risk factors for hernia recurrence. They can be repaired using an open approach or laparoscopically, with each method having its own risks, advantages, and disadvantages (4). As shown in this case, intraperitoneal mesh placement can be associated with severe complications, which may potentially be life threatening.

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Leptin is Involved in Activation of JAK-STAT3, PI3K-Akt, and SHP2-ERK1/2 Signaling in Gastric Cancer in the Upper Third of the Stomach: A Study Protocol

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Key words

proximal gastric cancer, obesity, leptin signaling

Study Protocol

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Abstract

Background. Our previous study showed that serum levels of leptin were significantly higher in patients with proximal gastric cancer compared to lower and mid-part gastric cancer. This might explain the higher incidence of proximal gastric cancer in obese patients. This study tests whether leptin induces activation of JAK- STAT3, PI3K-Akt, and ERK1/2 signal pathways in proximal gastric cancer.

Methods. Sixty-four gastric cancer patients operated on for gastric cancer with curative intent were included in the study. Tumor tissues and non-tumor tissues from the gastric antrum were retrieved from paraffin-embedded tissues of patients from the archive of the Institute of Pathology at the Maribor University Medical Center. The Qproteome FFPE Tissue Kit (Qiagen, Hilden, Germany) was used for extraction of proteins from the gastric cancer and antrum samples. Then Western blot analysis was performed to examine the effect of leptin on the activation of JAK-STAT3, PI3K-Akt, and ERK1/2 signaling pathways.

Conclusions. The results of our study provide insight into the underlying molecular mechanism by which leptin induces proximal gastric cancer. The findings indicate the importance of leptin as a mechanism in proximal gastric cancer in the overweight population.

Introduction

According to GLOBOCAN 2020 data, gastric cancer is the fifth most common and fourth most deadly cancer (1). Although the incidence of gastric cancer located in the antrum has been decreasing, the frequency of proximal gastric cancer has been increasing. Proximal gastric cancer has a worse prognosis, and the treatment is associated with higher morbidity (2).

The prevalence of proximal gastric cancer is higher in obese individuals, who, for some reason, are at an increased risk of developing cancer (3). Several mechanisms closely related to each other have been proposed to explain the association between increased body weight and proximal gastric cancer development, including increased intra-abdominal pressure, abnormal gastric motility, and bile reflux (4).

The effects of leptin, an essential adipokine secreted from fat tissue on proximal gastric cancer, has not been well investigated. Recently, studies have shown a link between obesity and carcinogenesis of gastric cancers via leptin-deregulated signaling pathways (5, 6). Leptin signaling is initiated by binding of leptin to its leptin receptor (ob-R), which regulates various signaling pathways in many human cancers. The main pathways governed by leptin are JAK2/STAT3 (Janus kinase 2/signal transducer and activator of transcription 3), PI3K/Akt (phosphatidylinositol-3-kinase/protein kinase B) and SHP2/ERK (Src homology phosphatase 2/extracellular signal-related kinase) (6). Activation of these pathways increases proliferation, inhibits apoptosis, and stimulates migration and invasion of cancer cells (7, 8). In gastric tissue, most of the cells expressing leptin receptors (Ob-R) are located in the lower gastric fundus (9). Furthermore, a recent study by us revealed that serum levels of leptin were significantly higher in patients with proximal gastric cancer compared to lower and mid-part gastric cancer (10). This might explain the higher incidence of proximal gastric cancer in obese patients. This finding supports the hypothesis that gastric cardia cancers may have a different tumor biology from non-cardia cancers (5).

This study investigates the role of leptin in proximal gastric cancer.

Methods

Study Design

This single-center retrospective study included 64 patients with gastric cancer. This study was approved by the Institutional Ethics Committee of the Maribor University Medical Center.

Study Population

The sixty-four patients with gastric cancer underwent surgery at the Maribor University Medical Center between April 2019 and August 2021. Patients eligible for study enrollment were worked up diagnostically with preoperative standard laboratory tests, a chest X-ray, an upper gastrointestinal endoscopy, and computer tomography of the chest and abdomen. Demographic, anthropometric, and clinical data were obtained for all patients. Patients eligible for perioperative treatment received four cycles of FLOT, consisting of docetaxel (60 mg/m^2), oxaliplatin (85 mg/m^2), leucovorin (200 mg/m^2), and 5-fluorouracil (2,600 mg/m^2 as a 24 hr infusion), all given on day 1 and performed every 2 weeks, and surgery followed 3 weeks after completion of chemotherapy. Patients received four cycles of FLOT postoperatively. Patients were operated on laparoscopically or open. All the patients included had participated in our previous study, in which blood samples were collected preoperatively from the patients to determine their preoperative serum leptin levels.

Tissue Block/Sections

This study retrieved paraffin-embedded tissues of patients with gastric cancer that had undergone a gastrectomy with lymphadenectomy at the Maribor University Medical Center between 2019 and 2021 from the archive of the Institute of Pathology at the Maribor University Medical Center. For all cases included, representative regions of adenocarcinoma and histologically normal gastric tissue from the antrum were marked by an experienced pathologist. From the marked tissue, two serial sections 10 µm thick were prepared with a microtome.

Deparaffinization and Protein Extraction

The Qproteome FFPE Tissue Kit (Qiagen, Hilden, Germany) was used for extraction of proteins from the gastric cancer and antrum samples. To remove the paraffin, two serial FFPE tissue sections of gastric cancer, 10 µm thick and with an area of 100 mm^2, were placed in Eppendorf tubes and incubated at room temperature in xylene for 10 min. This was followed by rehydration in a series of 100%, 96%, and 70% (v/v) ethanol. Each preparation step was performed in a collection tube (1.5 ml) with a 1 ml volume of the respective reagent. After adding 1 ml volume, the sample was vortexed vigorously for 10 s and incubated for 10 min at room temperature (25 °C), and then centrifuged in a micro-centrifuge for 10 min at full speed. The supernatant was discarded, and each preparation step was repeated twice using fresh reagent (11).

After deparaffinization and rehydration, 100 µl EXB extraction buffer (Qproteome FFPE Tissue Kit, Qiagen, Hilden, Germany) was added to the tube containing the tissue pellet. Then the tube was incubated on ice for 5 minutes and heated at 100 °C for 20 min on a heating block, and subsequently incubated at 80 °C for 2 h using a thermomixer with agitation at 750 rpm. Next, the tube was placed at 4 °C for 1 min and centrifuged for 15 min at 14,000 × *g* at 4 °C. The supernatant containing the extracted proteins was recovered (11).

SDS-PAGE and Western Blot

Equivalent amounts of protein extracts obtained from FFPE tissues were electrophoresed in 12.5% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were blocked with 5% non-fat milk in tris-buffered saline and then incubated with the antibodies phosphorylated Akt (phospho-Akt Ser473), phospo-STAT3, and phospho-ERK1/2. Signals were detected with the chemiluminescent method.

Statistical Analysis

All statistical analyses were performed with SPSS 22.0. The differences in continuous variables were analyzed using the Mann–Whitney *U* test, and the differences in categorical variables were analyzed using the chi-squared test. The expression of leptin and phospho- was compared in gastric cancer tissue and normal gastric tissue from the antrum using the chi-squared test and Fisher's exact test. Statistical significance was defined as $p < 0.05$.

Discussion

Obesity is a significant health concern in developed countries, with a dramatically increased incidence over the last decade. Obesity has been identified as a risk in many cancers, including gastric cancer. Hyperleptinemia is a common feature of obese patients (6). Our previous study suggested a correlation between obesity and proximal gastric cancer (10). Although white adipose tissue is the main site of leptin secretion, gastric tissues can also secrete a small amount. However, we believe that leptin plays a greater role (12).

The leptin activation of JAK-STAT3 and PI3K-Akt signaling has been studied extensively in various cancers. Briefly, the JAK-STAT and PI3K-Akt pathways play a crucial role in normal cellular processes, and aberrant activation modulates multistep development of gastric cancer, including autophagy, proliferation, inflammation, chemoresistance, and metastasis. Emerging evidence demonstrates that leptin (through the leptin receptor) can control the JAK-STAT and PI3K-Akt signaling pathways. The result is increased activation of STAT3 and Akt. Limiting STAT3 and Akt activity could help prevent malignancy. These findings suggest that targeting leptin signaling in gastric carcinoma may hold great potential as a novel therapeutic intervention for the treatment of patients with gastric cancer (7, 8, 13). However, the role of leptin activation of ERK1/2 pathways and its molecular signaling in gastric cancer has not been extensively evaluated. Various studies demonstrate that leptin enhances cell growth and inhibition of apoptosis by activating the MEK/ERK1/2 pathways in ovarian, endometrial, and prostate cancer (14–16).

We anticipate that, in proximal gastric cancer, phosphorylation of STAT3, ERK1/2, and Akt is a consequence of increased leptin production of adipose tissue. By demonstrating that the JAK-STAT, ERK1/2, and PI3K-Akt signaling pathways are implicated in the cell proliferative effect of leptin, we have emphasized a new role for leptin, linking proximal gastric cancer and obesity. However, as we also expected, phosphorylation of STAT3,

ERK1/2, and Akt in middle and distal gastric cancer and healthy gastric tissue can be activated by various ligands, shown though the careful interpretation of data. All our findings can help provide theoretical and experimental bases for further research on the etiology of proximal gastric cancer in obese patients.

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