Hyperbaric oxygen treatment for pelvic radiation-induced injuries

From a multicenter randomized controlled trial to an experimental cell model

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Alle Dinge sind Gift,
und nichts ist ohne Gift;
allein die dosis machts,
daß ein Ding kein Gift sei

- Filippus Aureolus Theophrastus Bombastus von Hohenheim
Also called Paracelsus 1493-1541

All things are poison
and nothing is without poison
only the dose makes
that something is not poison

- Filippus Aureolus Theophrastus Bombastus von Hohenheim
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ABSTRACT

Introduction Cancer is affecting a growing number of persons. Still, the treatment and survival of cancer is improving. Radiation therapy is used in the treatment of cancer. Late radiation-induced injuries afflict 5–15% of irradiated patients. The urinary bladder and bowel may be affected after irradiation of cancer in the pelvic region. Symptoms can be severe, with impaired health related quality of life (HRQoL). Hyperbaric oxygen therapy (HBOT) involves breathing oxygen at high ambient pressure. HBOT can reverse radiation-induced injuries, alleviate patient-perceived symptoms, and improve HRQoL.

We aimed to clarify the effects of HBOT on late radiation-induced injuries in the urinary bladder and bowel, and to clarify some of the underlying mechanisms through which HBOT exerts its effects.

Methods A prospective cohort study assessed effects of HBOT on patient-perceived symptoms (Paper I). A rat study assessed reversal of radiation-induced stress with HBOT (Paper II). A methodological experiment assessed reversal of HBOT on cellular death induced by radiation (Paper III). A multi-center, randomized, controlled trial assessed patient-perceived symptoms, HRQoL, and objective clinical outcomes (Paper IV).

Result HBOT can alleviate patient-perceived symptoms, reduce objective findings, and improve HRQoL in patients affected by late radiation-induced injuries (Paper I, IV). Oxidative stress and downstream effects, induced by the irradiation, can be reversed by HBOT (Paper II). Paper III outlines a method for studies on urothelial cells exposed to radiation and HBOT.

Conclusion HBOT can reduce radiation-induced oxidative stress and inflammatory response. HBOT can reverse injuries induced by radiation therapy to the pelvic region, alleviate patient-perceived symptoms and lead to improved HRQoL.

Keywords: Hyperbaric oxygen treatment, hyperbaric oxygen, late radiation-induced injury, cystitis, proctitis, reactive oxygen species, radiation therapy, quality of life

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SAMMANFATTNING PÅ SVENSKA

Risken att drabbas av cancer ökar med åldern och med ökande medellivslängd drabbas allt fler av cancer under sitt liv. Cancer är den främsta dödsorsaken i välbärgade länder som Sverige. Cancerbehandlingen blir samtidigt mer effektiv och fler personer lever allt längre efter att ha genomgått behandling mot cancer.

Cancer kan behandlas på flera olika sätt, beroende på tumörens lokalisation, allvarlighetsgrad och individuella faktorer. Ett vanligt sätt att behandla cancer på är genom strålning. Denna behandling kan ge upphov till biverkningar som kan bli symptomgivande flera år efter avslutad behandling. Sena biverkningar uppkommer till följd av skador på celler orsakade av fria syreradikaler. Dessa skador leder bland annat till kronisk inflammation, minskad kärlförekomst, med åtföljande lägre syrgasnivåer och ökad bindvävsomvandling av vävnaden.

Denna avhandling fokuserar på sena biverkningar efter strålbehandling mot cancer i bäckenregionen. Prostatacancer är den vanligaste cancerformen hos män. Gynekologisk cancer är vanligt förkommande hos kvinnor. Därtill kommer cancer i urinvägar och ändtarm som drabbar bägge könen. Prostatacancer drabbar ofta äldre män, medan de kvinnliga cancerformerna ofta drabbar personer i yngre åldrar. Fler förväntade levnadsår efter strålbehandling leder till att de som drabbas av sena biverkningar får leva fler år med sina besvär. Besvären blir vanligen mer och mer uttalade med åren.

Sena biverkningar efter strålbehandling av cancer i bäckenregionen inkluderar besvär från urinblåsan, ändtarmen och könsorganen. Av de som strålas mot cancer i bäckenregionen drabbas cirka 5–15% av uttalade besvär med stor inverkan på deras dagliga liv. Symtom från urinvägar och ändtarm inkluderar blödningar, täta trängningar, smärta, läckage och urinvägsstopp. I allvarligare fall kan framförallt blödningar göra att urinblåsan eller ändtarmen måste avlägsnas kirurgiskt.

Hyperbar syrgasbehandling är syre givet under tryck som är högre än det normala omgivande trycket. Denna behandling ges i en tryckkammare där man andas ren syrgas. Höga nivåer av syrgas leder till en ökad förekomst av fria syreradikaler. Dessa har inte någon påvisbar påverkan på frisk vävnad, men kan påverka en rad cellulära mekanismer i tidigare strålbehandlad vävnad. Inflammatoriskt svar kan dämpas eller helt släckas ut och nya blodkärl kan växa in i vävnaden.

Föreliggande avhandling undersöker, i fyra delarbeten, effekten av hyperbar syrgasbehandling efter strålbehandling av cancer i bäckenregionen. Majoriteten av patienterna som behandlades med hyperbar syrgas upplevde symtomlindring och förbättrad hälsorelaterad livskvalitet, jämfört med personer som inte fick behandling. Djurstudier bekräftade teorierna om att hyperbar syrgasbehandling kan motverka biverkningar efter strålbehandling av urinblåsa.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Hyperbaric oxygen treatment in radiation-induced cystitis: a prospective trial on patient-perceived quality of recovery**
Oscarsson N, Arnell P, Lodding P, Ricksten S-E, Seeman-Lodding H, *Int J Radiation Oncol Biol Phys, 87 (4), 670–675, 2013 doi:10.1016/j.ijrobp.2013.07.039*

II. **Hyperbaric oxygen treatment reverses radiation-induced pro-fibrotic and oxidative stress responses in a rat model**
Oscarsson N, Ny L, Mölne J, Lind F, Ricksten S-E, Seeman-Lodding H, Giglio D *Free Radical Biology and Medicine, 103, 248–255, 2017 doi: 10.1016/j.freeradbiomed.2016.12.036*

III. **Hyperbaric oxygen reverses radiation-induced cell death in human urothelial and endothelial cells – development of a cell model**
Oscarsson N, Podmolikova L, Seeman-Lodding H, Bergo M, Giglio D *(Manuscript)*.

IV. **Radiation-induced cystitis treated with hyperbaric oxygen (RICH-ART) – a randomized, controlled, phase 2-3 trial**
Oscarsson N, Müller B, Rosén A, Lodding Pär, Mölne J, Giglio D, Hjelle KM, Vaagbø G, Hyldegaard O, Vangedal M, Salling L, Kjellberg A, Lind F, Ettala O, Arola O, Seeman-Lodding H *Lancet Onc Epub 2019 Sep 16 doi: 10.1016/S1470-2045(19)30494-2*

CONTENT

ABBREVIATIONS

8–OHdG	8–oxo–2′–deoxyguanosine
ANOVA	Analysis of variance
AP–1	Activator protein 1
CXCR4	CXC chemokine receptor type 4
DNA	Deoxyribonucleic acid
EPC	Endothelial progenitor cells
EPIC	Expanded Prostate Index Composite
EUBS	European Underwater and Baromedical Society
FDA	Food and Drug Administration
GSH	Glutathione
HBOT	Hyperbaric oxygen therapy
HIF–1α	Hypoxia-inducible factor 1-alpha
HO–1	Hemeoxygenase one
HRQoL	Health-related quality of life
i/e/nNOS	intrinsic / endothelial / neural nitric oxide synthase
IFN–γ	Interferon gamma
IL-1, 2 etc.	Interleukin 1, 2
LENT/SOMA	Late effects normal tissue / subjective, objective, management, and analytic
MID	Minimal important difference
NF-κB	Nuclear-factor kappa-light-chain-enhancer of activated B cells
NK cell	Natural killer cell
NRF2α	Nuclear respiratory factor 2 alpha
PMN	Polymorf-nuclear (cells)
PTEN	Phosphate and teosin homolog
RICH-ART	Radiation-induced cystitis treated with hyperbaric oxygen – A randomized controlled trial
ROS	Reactive oxygen species
RTOG/EORTC	Radiation Therapy Oncology Group and European Organization for Research and Treatment of Cancer
SF-36	Short form 36
SFAI	Svensk förening för anestesi & intensivvård (Swedish Society for Anesthesiology and Intensive Care)
SOD	Superoxide dismutase
TGF–β	Transforming growth factor beta
TNF	Tumor necrosis factor
Txn	Thioredoxin
UHMS	Undersea and Hyperbaric Medical Society
VEGF	Vascular endothelial growth factor

DEFINITIONS IN SHORT

Hyperbaric oxygen therapy	Breathing oxygen at a higher than normal ambient pressure. In clinical practice, this usually means a partial pressure of oxygen of 200 kPa or higher.
Radiation therapy	Cancer treatment utilizing ionizing energy to kill cancer cells. External radiation often uses X-rays, but protons and other types of energy are also used.
Brachy therapy	A form of radiation therapy where a radioactive material is placed near the cancer.

1 INTRODUCTION

When hyperbaric oxygen therapy (HBOT) is mentioned, most people tend to think about scuba diving, if anything. While this doctoral thesis explores the use of HBOT, it has nearly nothing to do with scuba diving. Rather, it focuses on the effects that a higher than normal partial pressure of oxygen may have on irradiated cells and tissue, as well as on overall organ function and the general health of patients who have undergone radiation therapy and later developed radiation-induced injuries.

But the story begins with a misconception. With the knowledge that some bacteria were killed in an environment with high levels of oxygen. Robert E. Marx et al. tried to treat what was perceived as deep bacterial infections causing necrosis in previously irradiated bone with HBOT.[1] The treatment was successful, but it was later discovered that the necrotic areas were aseptic, i.e., the bacteria did not cause the necrosis, and thus HBOT did not exert its effect by killing bacteria, but rather through some other mechanisms.[2]

How can oxygen, a molecule from the periodic system, when delivered at a higher than normal ambient pressure, influence cells and tissue subjected to irradiation therapy several years earlier? How can these effects reverse changes observed in affected organs and alleviate patient-reported symptoms? Indeed, the links between oxygen, radiation therapy, normal cells, and cancer cells are intricate. This thesis aims to clarify some of these links and further elucidate the role of HBOT in late radiation-induced injuries.

The introduction starts with the history of HBOT and radiation therapy. This history is important for understanding the treatment's role in current clinical practice. It continues with a description of oxygen and radical oxygen species (ROS), which are key actors in this thesis. The mechanisms behind the development of cancer, the role of radiation therapy in its treatment, and the mechanisms at work in the development of late radiation-induced adverse effects are explained. This is the scene in which this thesis is played out. The use of hyperbaric chambers and a general description of the indications, dosage, and adverse effects of HBOT will follow. Lastly, the very essence of this thesis, HBOT for pelvic radiation-induced injuries, is the name of the play.

1.1 THE HISTORY OF HYPERBARIC OXYGEN THERAPY AND RADIATION THERAPY

Hyperbaric medicine usually constitutes a very marginalized part of medical school curricula. There is limited interest from the pharmaceutical industry in providing funding and supporting research in the field. Although the use of HBOT spans several medical specialties, it does not naturally fit into any of them. The hyperbaric chambers required to administer the treatment are not readily available and may be perceived as too costly.[3] Moreover, HBOT has been used for indications, such as autism, multiple sclerosis, cerebral palsy, that lack scientific support, which may have influenced its reputation negatively.[4-6] Together, this might explain the lack of large randomized controlled trials in the field of HBOT. There is, however, a long history of research in the field. A search with the term "Hyperbaric oxygen" in the US National Library of Medicine (2020-02-15) returned 10,813 papers (ncbi.nlm.nih.gov/pubmed/?term=hyperbaric+oxygen).

The histories of HBOT and radiation therapy share some similarities. Both entered clinical use in the beginning of the 20th century. Both were tested on nearly all known medical conditions. Initially, due to a lack of solid scientific evidence, the respective effects of both therapies were vastly exaggerated and occasionally used in ways that harmed or even killed patients and sometimes even doctors and nurses.[7]

1.1.1 ELEVATED PRESSURE

Air pressure was first measured (with a mercury barometer) and described in the 17th century by the Italian physicist Evangelista Torricelli.[8] The first documented use of a hyperbaric chamber dates from the same century, to 1662, when the British clergyman Nathaniel Henshaw built a system to elevate and decrease air pressure in a closed room, which he called a *domicilium*.[9] He claimed that acute conditions could be treated by elevating air pressure, i.e., *hyperbaric* treatment, and that chronic conditions could be treated by decreasing air pressure, i.e., *hypobaric* treatment.

The lethal physiology of atmospheric pressure became apparent during the construction of the Brooklyn Bridge in New York in the late 1800s. Footings were set in riverbeds and high-pressure tunnels were built to keep the water out. However, such pressure also dissolved nitrogen gas molecules in the blood of tunnel workers. When they emerged from the pressurized conditions, the gas came out of solution causing a life-threatening condition: decompression sickness. This condition killed about one-quarter of the workers. During the building of the Lincoln Tunnel under the Hudson River a few years later, decompression chambers were used to slow depressurization. Deaths related to decompression sickness dropped from 25% to almost 0.[10]

1.1.2 DISCOVERY OF OXYGEN

The Polish alchemist Michael Sendivoguis identified a substance in the air that he called "cibus vitae" (life's food) as early as 1604.[11] However, it took another century for oxygen to be described in a distinctive way, one that historically defined its true discovery. The English chemist Joseph Priestly and the Swedish pharmacist Carl William Scheele both discovered and described oxygen. Scheele conducted his experiment in 1772, but he did not publish it until 1777.[12] Priestly made and published his experiment in 1774.[13] The term *oxygen* (from Greek ὀξύς (oxys), meaning acid and -γενής (-genēs) meaning producer) was coined a few years later by Antoine Lavoisier, a French chemist.[14]

It took over a century from the discovery of oxygen for it to be implemented for clinical use in medicine. The lack of a technique to concentrate and store oxygen were the main reasons for this delay. The first documented administration of oxygen in a clinical setting was to a patient with pneumonia in 1885.[15]

1.1.3 HYPERBARIC CHAMBERS IN MEDICAL USE

In the 1830s, a few hyperbaric chambers were built. These early chambers used ambient air and were called "pneumatic chambers" or "compressed air baths". Hyperbaric chambers flooded with oxygen had already been tested at this time. Apart from the danger of handling oxygen and the risk of fires and explosions, reports stated that oxygen was toxic, causing convulsions and death, in concentrations of 300–500 kPa.[16] This halted the advancement of hyperbaric *oxygen* treatment for another half century.

The French physician Victor-Théodore Junod reported that hyperbaric therapy resulted in increased circulation in internal organs and the "production of feelings of well-being" as well as increased general health.[17] Junod treated conditions such as tuberculosis, cholera, deafness, and menorrhagia, and he reported successful results in many of these conditions.[18]

In 1872, Paul Bert, a French scientist, engineer, and physician, published *La Pression Barometrique*, in which he described the physiological effects of air under increased and decreased atmospheric pressure. In 1885, C. Theodore Williams published his "Lectures on the Compressed Air Bath and its Uses in the Treatment of Disease" in the *British Medical Journal*. He described the use of atmospheric air under different atmospheric pressures to treat diseases. He stated that this therapy was among the most important advances in modern medicine and expressed astonishment that it had thus far been ignored.

At the end of the 19th and beginning of the 20th century, several larger hyperbaric chambers were built. Some were even used as hotels or spas, and hyperbaric medicine was marketed as "the universal treatment of all disease."[19] Other

chambers were used as operating rooms, and still others were used as hospital rooms, predominately for patients with pulmonary disease.

It was in one of these chambers, in Lawrence, USA, that Orville J. Cunningham first treated patients with influenza during the late 1920s. He reported great improvements for these patients, especially those who had been admitted in a cyanotic or comatose state.[20] The chamber was, however, abruptly closed after a mechanical failure caused the complete loss of pressure, killing all the admitted patients.

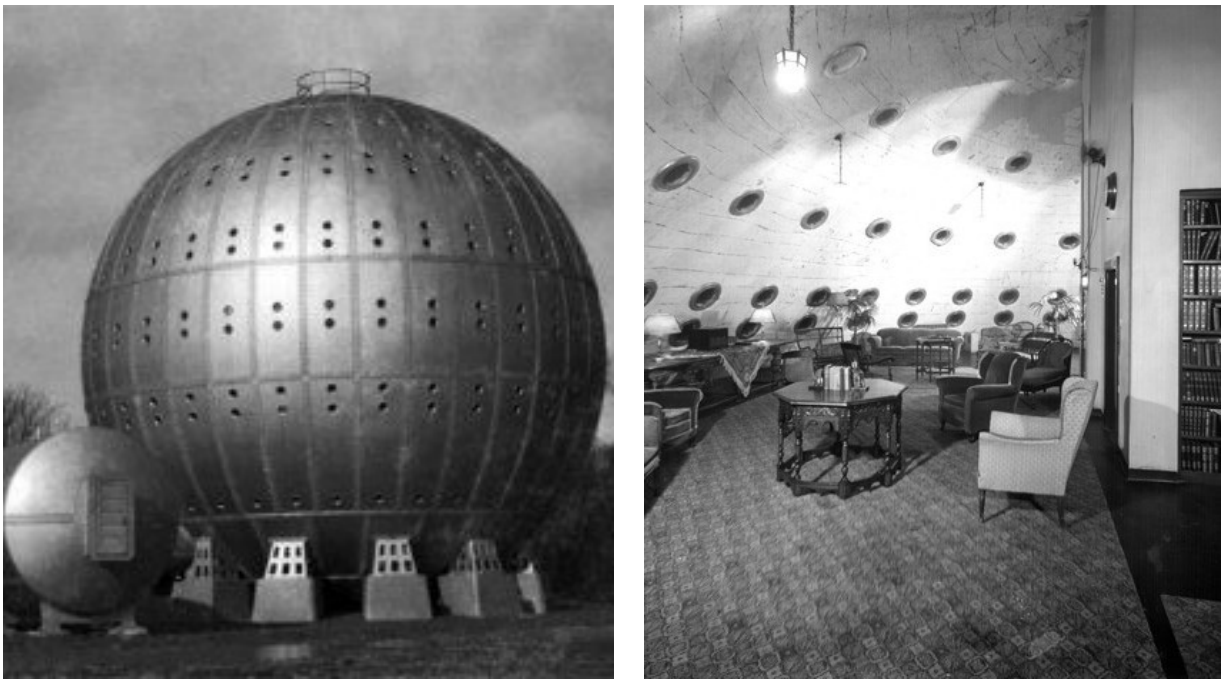

Figure 1. Cunningham Sanitarium, a 65-foot steel sphere, with a capacity of 40 patients. It used compressed air, not pure oxygen. It was in use for just over a decade before it was dismantled and scrapped.
Image Courtesy of Cleveland State University. Michael Schwartz Library. Special Collections.

Cunningham believed that anaerobic infections played a central role in the development of cancer, syphilis, hypertension, diabetes, and many other diseases. He was also convinced that hyperbaric medicine could eradicate anaerobic bacteria and hence cure many major diseases.[20] Thus, he persisted in his work, building the largest hyperbaric chamber in the world in 1928. Situated in Cleveland, Ohio, USA, this hyperbaric hotel was five stories high, and each room was fully furnished (Figure 1). Cunningham was, however, reluctant to publish any scientific evidence on the medical effects of the treatment. The American Medical Association demanded proof, but when they failed to receive it, they made the following announcement: "*Under the circumstances, it is not to be wondered that the Medical Profession looks askance at the 'tank treatment' and intimates that it seems tinctured much more strongly with economics than with scientific medicine.*"[21] After just a decade, the chamber was closed and later dismantled.

1.1.4 DIVING MEDICINE PAVED THE WAY

In parallel with the dangers of tunnel work, the risk of developing decompression sickness is a major concern in relation to underwater activities, where the pressure is also elevated. Navies around the world have used hyperbaric chambers to treat decompression sickness. This condition occurs when the ambient pressure is decreased, thus causing gas dissolved in the tissue to be released in the same manner as when a bottle of carbonated liquid is opened. If the pressure of the gas is high enough, it can be released from the solution and form bubbles that may occlude blood flow or exert pressure on nerves and other tissues. If the patient is recompressed, i.e., the ambient pressure is increased again, e.g., in a hyperbaric chamber, then the gas will be forced back into the solution and the bubbles will disappear.

Another concern in relation to underwater activities is aforementioned oxygen toxicity. Although Dräger had already constructed pressurization protocols with tolerable levels of oxygen in 1917, it took another 20 years before these protocols were implemented in clinical practice. In 1937, Albert Richard Behnke and Louis Shaw, two physicians working for the US Navy, administered the first hyperbaric *oxygen* treatment to a patient suffering from decompression sickness.[22]

1.1.5 HYPERBARIC OXYGEN THERAPY IS BORN

Alvaro Ozorio de Almeida was the first to use HBOT for conditions other than diving-related injuries. He treated leprosy, cancer, and gas gangrene during 1934–1941.[23]At the beginning of the 1950s, a Dutch cardiac surgeon, Ite Boerema, conceived the idea of flooding the body with oxygen before cardiac and pulmonary surgery. After a few successful operations on animals, he convinced the University of Amsterdam to build a large operating hyperbaric chamber.[23,24] Boerema performed successful cardiac and pulmonary surgery in the chamber for two decades, and similar chambers were built in Sydney, Boston, and in many other university hospitals. These hyperbaric operating chambers gradually became obsolete when techniques for extracorporeal circulation were developed.

Boerema also published a paper called “Life Without Blood,” in which he demonstrated that life could be sustained practically without any hemoglobin, if the levels of dissolved oxygen in plasma were sufficiently high.[25] This could be achieved by the administration of HBOT. In 1961, Boerema also successfully treated clostridial myonecrosis (gas gangrene) with HBOT, thus illustrating the bacteriocidic effect of high levels of oxygen on anaerobic bacteria.[26] Boerema has been credited with the title, “Father of modern-day hyperbaric medicine.”[27]

Churchill-Davidson, from Great Britain, performed a series of experiments on humans that involved the application of HBOT to increase the sensitivity of

malignant tumors to radiation therapy.[28] Radiation therapy was conducted through a window in the hyperbaric chamber, and the patients were heavily sedated to prevent oxygen toxicity-induced seizures.[29] In 1966, Churchill-Davidson summarized his work, concluding that "Early treatment results are extremely encouraging."[30] In later studies, a few cancer types, such as glioblastoma and sarcoma in the head and neck region, were treated with a combination of the two therapies, leading to reduced mortality and fewer recurrences of cancers compared to radiation alone.[31] However, adverse events and late radiation-induced injuries were more common for patients who have received combined therapy.[31] There were also great risks involved with administrating radiation to sedated patients in a hyperoxic environment, e.g., fires and explosions, aspiration of gastric content, and convulsions. Hence, the combination of the two therapies never became widespread.[23] More recent research in this area are summarized in "Hyperbaric oxygen therapy and cancer—a review".[32]

The fact that HBOT could act as an antibiotic agent led the oral and maxillofacial surgeon Robert Marx to explore whether HBOT could be used to treat osteoradionecrosis.[1] This condition predominantly develops after radiation therapy for cancer in the orofacial area and was once believed to be partly caused by chronic bacterial infection.[33] In 1981, Marx et al. showed that HBOT improved osteoradionecrosis, but paradoxically, he also showed that the condition was not caused by chronic infection. Osteoradionecrosis is an aseptic necrosis caused by radiation, with hypoxic-hypovascular-hypocellular tissue and, eventually, chronic non-healing wounds.[2]

The new causality of osteoradionecrosis called for another explanation for why HBOT seemed to be beneficial for treating this condition. Marx continued his work and published a number of reports during the early 1980s.[1,2,34-38] He showed that HBOT stimulates the growth of new blood vessels in previously irradiated and necrotic bones. His findings are summarized in "A New Concept in the Treatment of Osteoradionecrosis,"[2] a paper which paved the way for the treatment of late radiation-induced injuries with HBOT. More recent studies are summarized in section 1.6.

1.1.6 A NEW KIND OF RAY

It only took three years from the discovery of the so-called "X-ray" in 1896 for it to be clinically administered in the treatment of cancer. The unknown source of the ray made the German physics professor, Wilhelm Conrad Röntgen, call it "X-ray," although his surname, "Röntgen," is frequently used as a synonym.[39] In 1901, Röntgen was awarded the Nobel Prize in Physics for his discoveries.

Within a few years, several well-known scientists, such as Marie and Pierre Curie, Henri Becquerel, and Ernest Rutherford, added more knowledge to the field.[40] The use of radiation started as a diagnostic method, utilizing electromagnetic rays in relatively low-voltage machines. It continued with repeated application and increasing voltage and with radiation from radium, with cancer being only one of the many conditions to which it was applied (Figure 2).[41]

Figure 2. Radiation was marketed to treat all kinds of disease. "A century of x-rays and radioactivity in medicine: with emphasis on photographic records of the early years." Francis Mould (1993).
Public Domain: https://en.wikipedia.org/w/index.php?curid=32468063 (2019-10-05)

For some tumors, like carcinoma and epithelioma, radiation therapy seemed to be much more effective than other treatment modalities, which by that time mainly involved surgery.[226] Radium was also used to treat tuberculosis, arthritis, gout, sexual disorders, obesity, high blood pressure, and many other conditions.[42,43] In the early years, the positive effects of the treatment were exaggerated, and radiation therapy was marketed as the "universal treatment of all disease." The most famous "Radium SPA Hotel" was in St. Joachimsthal, where radon inhalation rooms and baths were available.

Without any other means to measure the radiation dose, radiologists tested radiation beams on their own arms. A dose that produced a pink skin reaction (erythema) was considered an optimal dose. Many of these radiologists later died of leukemia. The paradoxical finding that radiation therapy could not only cure cancer but also cause it launched (ongoing) efforts to refine the therapy and minimize its adverse effects.[44,45]

With advances in the technical field and improved knowledge of radiation and the response of tumor cells to irradiation, it became possible to target the tumor more accurately. With higher precision, the efficacy of the treatment improved, and adverse effects became manageable. Modern radiation therapy uses high-resolution images to map the tumor in three dimensions and target it from several different directions.[46,47]

1.2 OXYGEN, PRESSURE, AND REACTIVE SPECIES

The word *stress* can be used to describe conditions of imbalance between demands and resources. Oxidative stress can occur when the demand for oxygen exceeds the available oxygen (hypoxia), triggering an array of downstream effects. Oxidative stress can also occur when the available oxygen exceeds the demand (hyperoxia), paradoxically triggering similar downstream effects. While this might seem like a flaw in evolution, one must remember that hypoxia is a normal physiological state, while hyperoxia is not.

1.2.1 OXYGEN

Oxygen is a highly reactive agent that needs constant replenishment by photo-synthesis.[48] Aerobic organisms use oxygen for energy production, and most molecules in living organisms contain oxygen, e.g., proteins, carbohydrates, fats, nucleic acids, teeth, and bone.

Otto Warburg was awarded the Nobel Prize in 1931 for showing that oxygen is part of an enzymatic process in the mitochondria that conveys energy to the cells. Later, in 1938, Corneille Hayman was awarded the same prize for demonstrating that the levels of oxygen in the blood can be sensed by the carotid body, and that this sense is coupled with the regulation of breathing. In 2019, the Nobel Prize in Medicine was awarded to William G. Kaelin Jr., Sir Peter J. Ratcliffe, and Gregg L. Semenza for "their discoveries of how cells sense and adapt to oxygen availability," and how this affects angiogenesis. The level of oxygen in and around the cell plays a fundamental role in cell function and gene expression. The regulation of metabolism, angiogenesis, the immune system, and the production of red blood cells are some examples of actions coupled with oxygen levels.[49]

Consequently, oxygen is contemporaneously used as a drug in many medical situations in order to treat regional or general hypoxia.

1.2.2 PRESSURE AND OXYGEN

Atmospheric pressure varies with elevation over sea level and current weather conditions. The standard atmosphere is defined as 101.325 kPa (1.01325 bar, 760 mmHg, or 14.696 psi). Pressure can be expressed as *absolute pressure*—in which case, it is measured from absolute zero = vacuum. Pressure can also be expressed as *relative pressure*—in which case, it is expressed as over or under the normal ambient pressure, i.e., the ambient pressure is used as a relative zero, and deviations from relative zero are expressed as over or under pressure.[48]

Ambient pressure decreases by around 10 kPa per 1000 meters of elevation over sea level. Water is denser than air, and hence the pressure is increased more rapidly when submerged; around 10 kPa per 1 meter of sea water. This means that the absolute pressure is doubled (200 kPa) at 10 meters of seawater.[48]

The partial pressure of a gas is the notional pressure of that gas if it occupies the entire volume alone and as a measurement of the thermodynamic activity of the molecules of the gas. The total pressure of gases in a mixture is the sum of all their respective partial pressures. Gases diffuse, dissolve, and react according to their partial pressures. The amount of oxygen necessary for respiration, and the amount that is considered toxic, is thus dependent on partial pressure and not concentration.[48]

The content of oxygen in normal air is around 21%, while 78% consists of nitrogen. The remainder is a mixture of several gases, such as carbon dioxide and inert gases. Each breath of fresh air is mixed with residual gases in the airway system, creating the so-called dead space ventilation. The residual gas is higher in carbon dioxide and humidity, and lower in oxygen. This dilution means that the pressure of oxygen in the alveoli is between 13–15 kPa, i.e., 6–8 kPa lower than in inspired air.[48]

1.2.3 OXYGEN CONTENT IN BLOOD

Gas exchange takes place in the lungs, where oxygen diffuses over the membrane of alveolae and into the blood. There is an additional 2-3-kPa drop in oxygen pressure during this passage, and the pressure of oxygen in the blood as it leaves the lungs is about 10–12 kPa.

Oxygen is bound to hemoglobin and dissolved in plasma. The cells extract oxygen from the blood in the capillaries. The cells require a steady delivery of oxygen and are dependent on constant blood flow as well as a sufficient content of oxygen in the blood. During normal resting conditions, the oxygen content of arterial blood is in the range of 13–18 ml/dl. The body can compensate for lower oxygen content with increased cardiac output and the redistribution of blood flow, but without adaptation, oxygen levels lower than 8-9 ml/dl will give rise to hypoxic cells.[48]

In the human body, most oxygen is bound to hemoglobin, and only a small fraction is dissolved in plasma. The formula for oxygen content in arterial blood is as follows:

$$CaO2 = (K \times [Hb] \times SaO2) + (0.023 \times PaO2)$$

CaO_2 *oxygen content in arterial blood*
K *constant for volume of oxygen bound to 1 gram of saturated haemoglobin (ml/g); 1.34 is used in these calculations*
[Hb] *concentration of haemoglobin (g/dl)*
SaO_2 *percentage of haemoglobin saturated with oxygen (%); 100% is used in these calculations*
0.023 *solubility coefficient of oxygen in plasma (ml/dl/kPa)*
PaO_2 *partial pressure of oxygen dissolved in arterial blood (kPa)*

During normal conditions, the content of oxygen is approximately:

$$1.34x13x1.00 + 0.023x12 = 17.42+0.3 = 17.45 \text{ ml/dl}$$

The saturation of hemoglobin is normally around 100% and can thus not be elevated further with additional inspired oxygen. Hence, only the oxygen dissolved in plasma can contribute to a higher relative pressure of oxygen in the tissues and cells. The content of inspired oxygen can be elevated to 100 kPa during *normobaric* conditions, which can yield an oxygen level of approximately 88 kPa in the alveoli. The content of oxygen will thus be:

$$1.34x13x1.00 + 0.023x88 = 17.42+2.024 = 19.44 \text{ ml/dl}$$

HBOT is defined as breathing oxygen at higher than normal ambient pressures. Compared to normal breathing, the oxygen content in the blood is elevated by around 10% (1.7 mg/dl) when breathing 100% oxygen at normobaric pressures and around 30% (5.2ml/dL) during HBOT given at a 240 kPa (absolute):

$$1.34x13x1.00 + 0.023x228 = 17.42+5.25 = 22.67 \text{ ml/dl}$$

When Boerema published his paper, “Life Without Blood,”[25] he used a 300 kPa (absolute) and 100% oxygen, and he diluted the hemoglobin of pigs to 0.6–0.2% (g/l not included in the paper). The content of oxygen in the blood was thus, theoretically:

$$1.34x2x1.00 + 0.023x288 = 2.68+6.624 = 9.30 \text{ ml/dl}$$

This level is above the hypoxic threshold, which means that the cells obtain enough oxygen necessary to survive, and hence it was proven that life could be sustained without nearly any hemoglobin.[25]

1.2.4 OXYGEN TOXICITY

Renaissance physician Paracelsus noted: “All things are poison, and nothing is without poison, only the dose permits something not to be poisonous.” However, before oxygen becomes poisonous, it may create several different effects that vary with the dose, i.e., the amount of oxygen molecules inhaled with each breath and the duration of the treatment. The effects of oxygen also differ between organs and between normal and pathologically changed tissues and cells.

Pulmonary fibrosis, retinopathic conditions, and renal, cardiac, and hepatic damage are some of the changes seen after longer periods of hyperoxia, even during normobaric conditions.[48,50-52] Hyperoxia can give rise to vasoconstriction with increased workload on the part of the heart. Due to the risk of complications, the use of oxygen in medical emergencies and intensive care should be carefully titrated.[53,54]

Pronounced hyperoxia can give rise to acute, adverse neurological effects, in which both partial pressure and duration of exposure exert toxic oxygen effects on the central nervous system. Symptoms include disorientation, rigidity, twitching, and generalized seizures, accompanied by the loss of consciousness.[55] There is a wide intra- and interindividual variation of exposure time before the onset of symptoms. Partial oxygen pressures exceeding 250 kPa usually give rise to acute neurological symptoms, but some individuals might develop symptoms at much lower levels (~160 kPa).[55] However, this also means that oxygen toxicity with seizures and unconsciousness can only occur during hyperbaric conditions.

The effects from hyperoxia can thus occur after longer periods of exposure to oxygen at relatively low partial pressures, while other effects can occur after shorter exposure periods but at much higher partial pressures. Both these aspects, partial pressure and time, are integral to the development of adverse as well as desirable effects.

1.2.5 REACTIVE OXYGEN SPECIES – ROS

Reactive oxygen species (ROS) is a collective term for many different oxygen derivates, each of which is reactive and unstable. ROS are produced as a by-product during normal metabolism in the mitochondria, but they can also be produced by many other chemical processes and external agents, such as radiation and hyperbaric oxygen (Figure 3).[56] These short-lived molecules and atoms are essential for many biological processes and act as obligate second messengers, but they can also interfere with and have deleterious effects on normal cellular processes.[57] The effects of ROS depend on an array of factors, such as the sites for ROS production, the type and amount of ROS molecules, and the levels and actions of counteracting systems.[56]

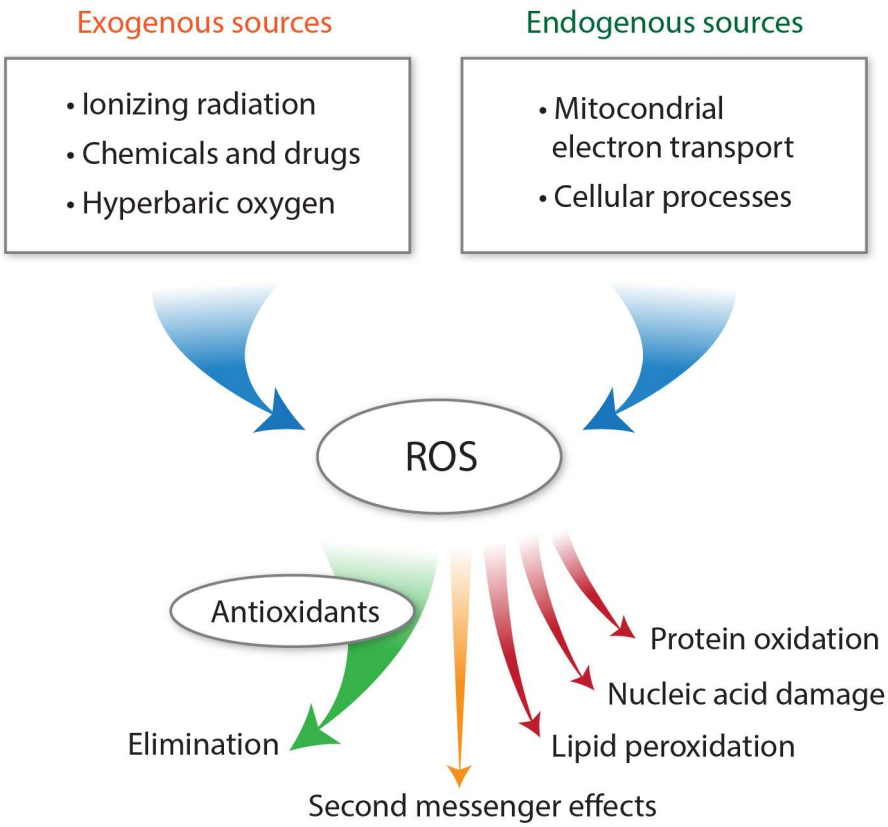

Figure 3. Reactive oxygen species (ROS) can be formed via exogenous or endogenous sources. Antioxidants neutralize ROS. Damage to lipids, proteins, and DNA might occur if the levels of ROS exceed the capacity of the antioxidative system.

The intricate balance between the production and elimination of ROS in the cells is partly maintained via the molecules responsible for eliminating ROS. Antioxidant enzymes, such as superoxide dismutase one and two (SOD–1, SOD–2), hemeoxygenase one (HO–1), glutathione (GSH), and thioredoxin (Txn), as well as exogenous antioxidants, neutralize ROS and uphold the so-called redox balance.[58] Elevated ROS levels activate gene transcription factors, such as nuclear factor erythroid 2-related factor 2 (NRF2) and nuclear respiratory factor two alpha (NRF–2α). These factors interact with the antioxidant response element (ARE) in the cell nucleus, which leads to an upregulation of genes that encode SOD–1, SOD–2, HO–1, GSH, catalase, and peroxidases.[56,59,60] Thus, a feedback loop is created, one that seeks to maintain a steady state of ROS.

Increased levels of ROS also lead to an upregulation of activator protein-1 (AP-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and MAP kinases.[56] These also induce antioxidative responses, but more importantly, they change the cellular state and can induce senescence and apoptosis.[61]

ROS can also activate various tumor suppressor genes, e.g., retinoblastoma protein (Rb), *p53*, phosphatase and tensin homolog (PTEN) and ROS are needed in the progression of the cell cycle to interact with growth factors and the tyrosine kinase

receptor.[62] ROS regulate the expression of some inflammatory cytokines, such as interleukin 1 and 6 (IL-1, IL-6), tumor necrosis factor (TNF), and transforming growth factor beta (TGF–β).[63] There is a close relationship between ROS, chronic inflammation, and fibrosis.[63,64]

One specific condition that leads to increased oxidative stress is hypoxia.[65] Hypoxia culminates in an increase of mitochondrial ROS that activate hypoxia-inducible factor 1-alpha (HIF-1α) and vascular endothelial growth factor (VEGF), which are key mediators of angiogenesis.[66,67] New blood vessels aim to counteract the hypoxia that the cells are sensing, thus constituting a vital signal pathway.

ROS also play a central role in cancer cells. ROS levels in cancer cells are elevated, mainly due to increased metabolism and mitochondrial malfunction, but also by some oncogenes, such as *Kras* and *C-myc.*[68,69] Hypoxia may also be present in fast-growing tumors due to hypoperfusion, which also generates increased ROS production.[70] Other processes, such as integrin activation and changed signaling in metastatic cancer, also result in increased ROS production.[71]

Oxidative stress causes mutations in the DNA. One mutation that is possible to detect is the modified DNA base 8-Oxo-2'-deoxyguanosine (8–OHdG).[72] This molecule can be used as a marker for the amount of oxidative stress to which a cell or tissue has been subjected.[56]

Thus, it can be concluded that the effects of ROS on cells may be vital, beneficial, harmful, or detrimental depending on a multitude of factors, such as the cell type, the ROS involved, counteracting systems, and physiological conditions. The duration and level of elevation of ROS also play an important role,[56] as shown in Figure 4.

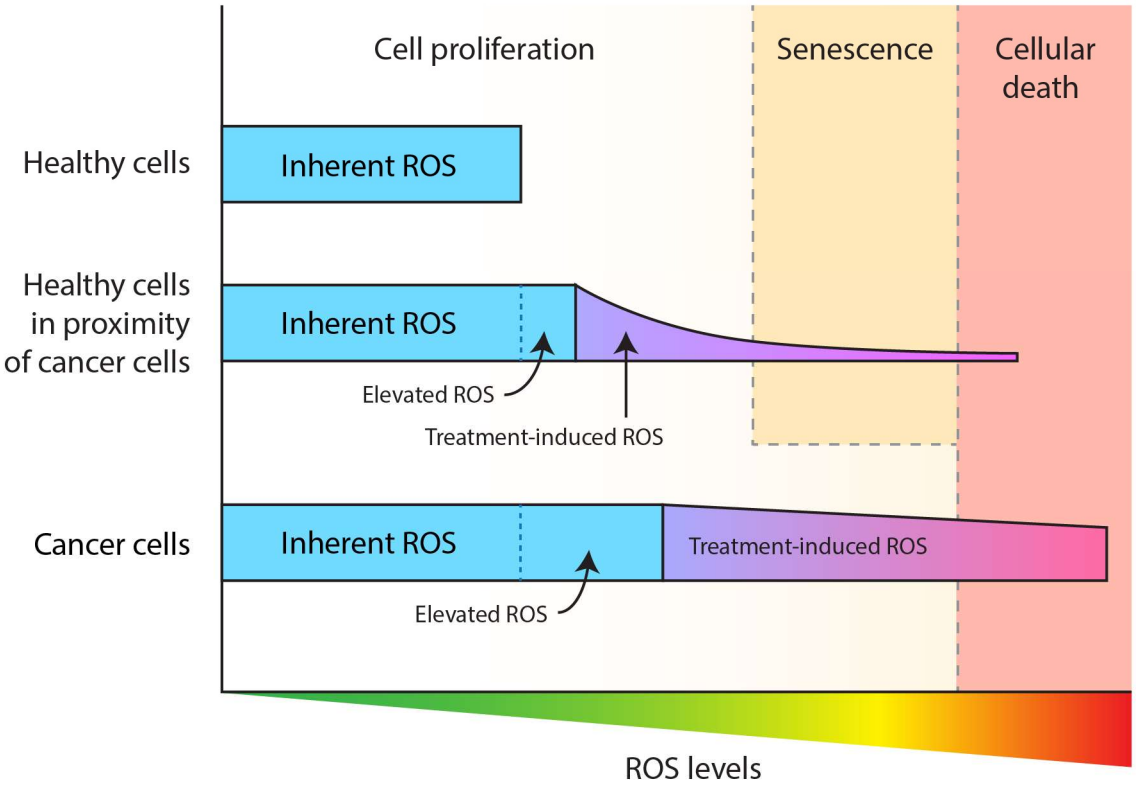

Figure 4. Healthy cells control their levels of ROS via the antioxidative system. Healthy cells in the vicinity of tumor cells may have elevated levels of ROS due to stress, inflammation, and competition of oxygen and metabolites. Radiation therapy aims to elevate the ROS levels in the cancer cells to lethal levels. Previously healthy cells might also die or enter senescence.

1.2.6 NITRIC OXIDE AND NITRIC OXIDE SYNTHASE

Nitric oxide is also an ROS that is involved in several physiological and pathological processes, such as vasodilation, immunological response, neurotransmission wound repair, and tumor development.[73] Nitric oxide is endogenously synthesized by nitric oxide synthases (NOS) that convert L-arginine to L-citrulline to nitric oxide. There are different kinds of NOS, i.e., inducible NOS (iNOS), endothelial NOS (eNOS), and neural NOS (nNOS).[73] iNOS is controlled at the gene transcription level, whereas eNOS and nNOS are controlled by intracellular processes.[74] During hypoxia, eNOS is upregulated and more nitric oxide is produced, which is coupled with the increased expression and activity of HIF–1α and VEGF.[75] Nitric oxide is involved in cell recruitment and vascular adhesion molecule expression during angiogenesis.[76]

The level of neural nitric oxide is increased during HBOT due to augmentation of nNOS caused by oxidative stress.[77] However, the production of nitric oxide is reduced in the airways when exposed to high partial pressures of oxygen.[78] HBOT

decrease iNOS activity in patients with diabetic ulcers via phosphorylation of NF–κB subunit *p65*.[79] However, several sessions of HBOT culminate in increased levels of nitric oxide in diabetic wounds, which is believed to be an important mediator of the effect of the treatment.[80] Although the production of nitric oxide is not directly dependent on the availability of oxygen, it is tightly regulated via feedback loops, and supranormal partial pressures of oxygen induce nitric oxide-dependent pathways involved in angiogenesis.[81]

1.3 CANCER AND RADIATION THERAPY

1.3.1 DNA – THE GENETIC CODE

Nearly all cells have a nucleus that contains most of the genetic code, i.e., deoxyribonucleic acid (DNA). These double-stranded molecules, called polynucleotides, are composed of two different pairs of single nucleotides: cytosine and guanine, and adenine and thymine. The nucleotides are stabilized by hydrogen bonds. Human DNA is arranged in 23 pairs of chromosomes and consists of around three billion base pairs. Only 1.5 percent of human DNA carries relevant information for protein coding; the remaining DNA is non-coding, although many of these sections play important roles in the regulation and expression of the genome.[82]

1.3.2 THE CELL CYCLE

The normal state of most cells is called the resting stage or growth stage zero (G0). The "resting" refers to the fact that the cell is not yet preparing to divide but is instead carrying out all its functions in the body. Some cell types stay in the resting stage for hours, others for years. The cell can enter the next stage, called G1-phase, in response to different growth stimuli. During this phase, the cell produces many proteins and molecules that will replicate the DNA. During the next phase, the S-phase, the DNA is replicated, a process in which errors in the DNA are likely to occur. The last stage is the G2-phase, which take place before cell division, called mitosis. The G1- and G2-phases represent important checkpoints that, if they fail, might stop and revert the division process or even kill the cell (Figure 5).[82]

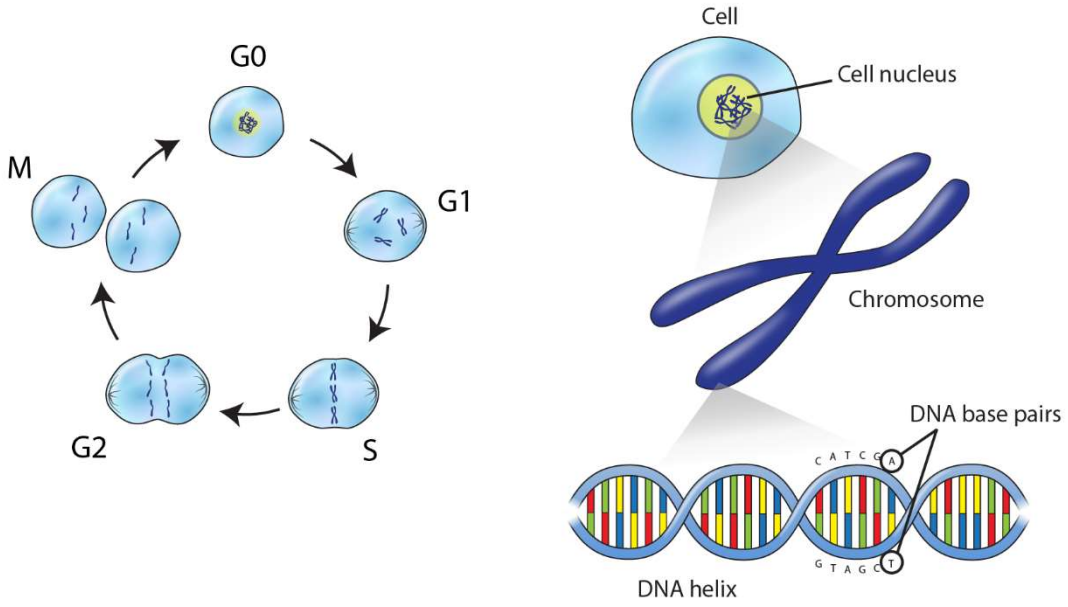

Figure 5. The cell cycle contains several steps during which the DNA is replicated. There are several checks for errors at each step in the cycle.

1.3.3 DEVELOPMENT OF CANCER

Although both intricate and advanced, cellular control and repair mechanisms are not able to correct all errors. Uncorrected errors are called *mutations* and are propagated down to each new generation of cells. Moreover, numerous extracellular agents can cause mutations in DNA, even when the cell is not dividing.[82]

If mutations lead to impairment in the cell, it is usually sensed, and the cell accordingly initiates a pathway that will either lead to its own death, apoptosis, or prevent it from growing or dividing into new cells, senescence. If the mutations are more severe, the cell might not even be able to initiate apoptosis and will thus be killed in an uncontrolled way, called necrosis (Figure 6).[82]

The cell might start dividing uncontrollably if the mutations occur in a part of the DNA responsible for the growth and division of the cell, or in areas that are responsible for the control and correction of errors. In this case, the cell will become a cancer cell. Most cells that develop into this dangerous state of relentless division are recognized as faulty by other cells, such as natural killer (NK) cells, macrophages, and cytotoxic T cells.

In the rare event that all these mechanisms fail, just one cancer cell can generate millions of daughter cells, forming both solid tumors and circulating cancer cells (Figure 6). The lack of sensing and response causes the cancer cell to disregard external factors that would normally have halted or stopped its division, such as low oxygen tension or acidic conditions.[82]

The severity of the cancer will depend on several factors, such as the type of original cell, its location in the body, the production, excretion, and expression of certain proteins and molecules, the speed of growth, and whether the cell respects normal tissue boundaries. If it does *not* respect these boundaries, thereby infiltrating other tissues and organs, the cancer cell is defined as malignant.[82] The severity of the cancer is also highly dependent on the overall state of the body in which it resides. Both cancer-specific characteristics and individual factors must thus be considered in the treatment of cancer.[83]

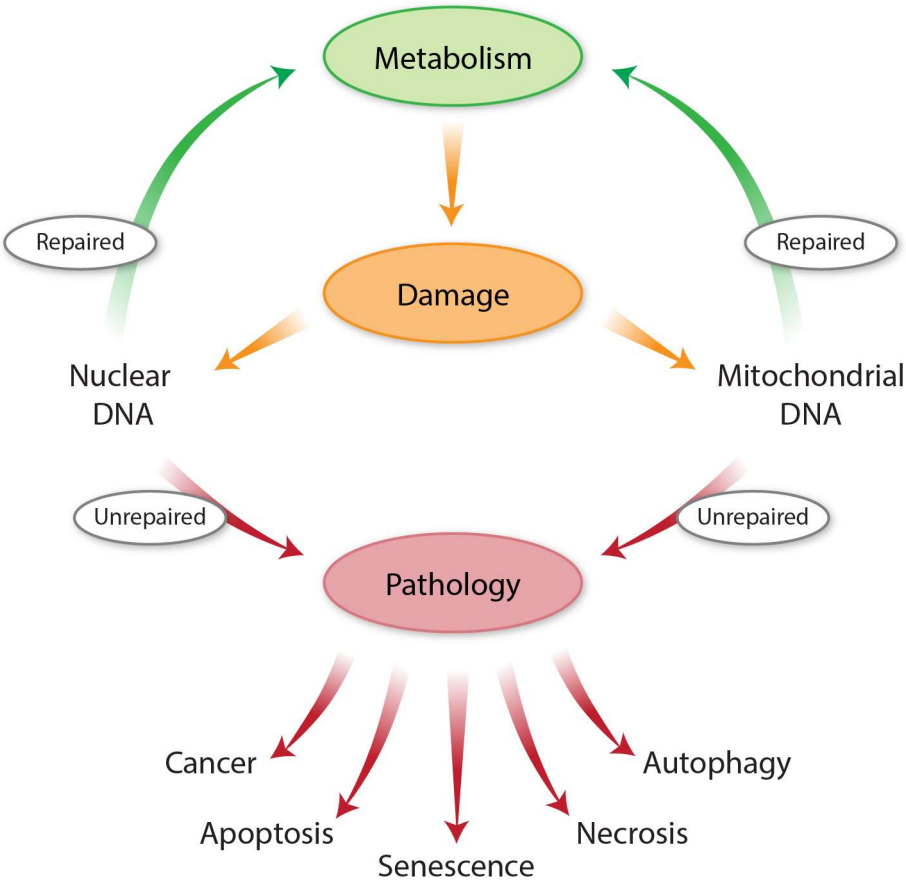

Figure 6. Cells are constantly subject to damage, with as much as 500,000 DNA modification events per cell per day. Most of these errors are corrected, and the cell continues to live and divide. If unrepairable errors are detected, the cell usually kills itself via apoptosis, but it can also enter senescence. Cancer cells are usually detected and killed by other cells via autophagy, but they occasionally give rise to tumors.

1.3.4 RADIATION THERAPY

The treatment of cancer can be divided into three different modalities: surgical removal, radiation therapy, and medical treatment (predominately chemotherapy, hormone therapy, and immunotherapy). The common goal is to effectively destroy cancer cells while, at the same time, doing as little harm to the patient as possible. Also, it is preferable for the treatment to be as fast and inexpensive as possible.[83]

There are different kinds of radiation: acoustic, electromagnetic, gravitational, photon, and particle. The clinical term *radiation therapy* refers to the use of particle or photon radiation with an energy level high enough to alter the state of other atoms, i.e., ionizing capabilities. The energy needed to classify radiation as ionizing is usually set to >10 eV. Examples of particle radiation include electrons, protons, neutrons, alpha particles, and beta particles. Photon radiation clinically uses X-rays and gamma rays.[84]

The energy that ionizing radiation carries can force single electrons out of their track around an atom, thereby leaving the atom with a net positive charge. Both the free electron and the atom become reactive, i.e., they disturb the stability of other atoms and molecules. Ionizing radiation can be directly lethal to the cell in higher doses, but radiation therapy is given in sub-lethal doses in order to conserve the healthy tissue surrounding the tumor. The presence of normal cells around the cancer cells limits the strength of the radiation dose that can be delivered in clinical practice.[84]

In clinical doses, it is not the radiation beam per se that causes the most damage to the cells, but rather the formation of ROS that are created in its wake. These highly reactive molecules can cause grave damage to the DNA and to the rest of the cell.[85] These effects are most apparent during cell division, when they can lead to one of three major pathways of cellular death: apoptosis, necrosis, or autophagy. All these pathways are dependent on ROS (Figure 7).[61,86,87]

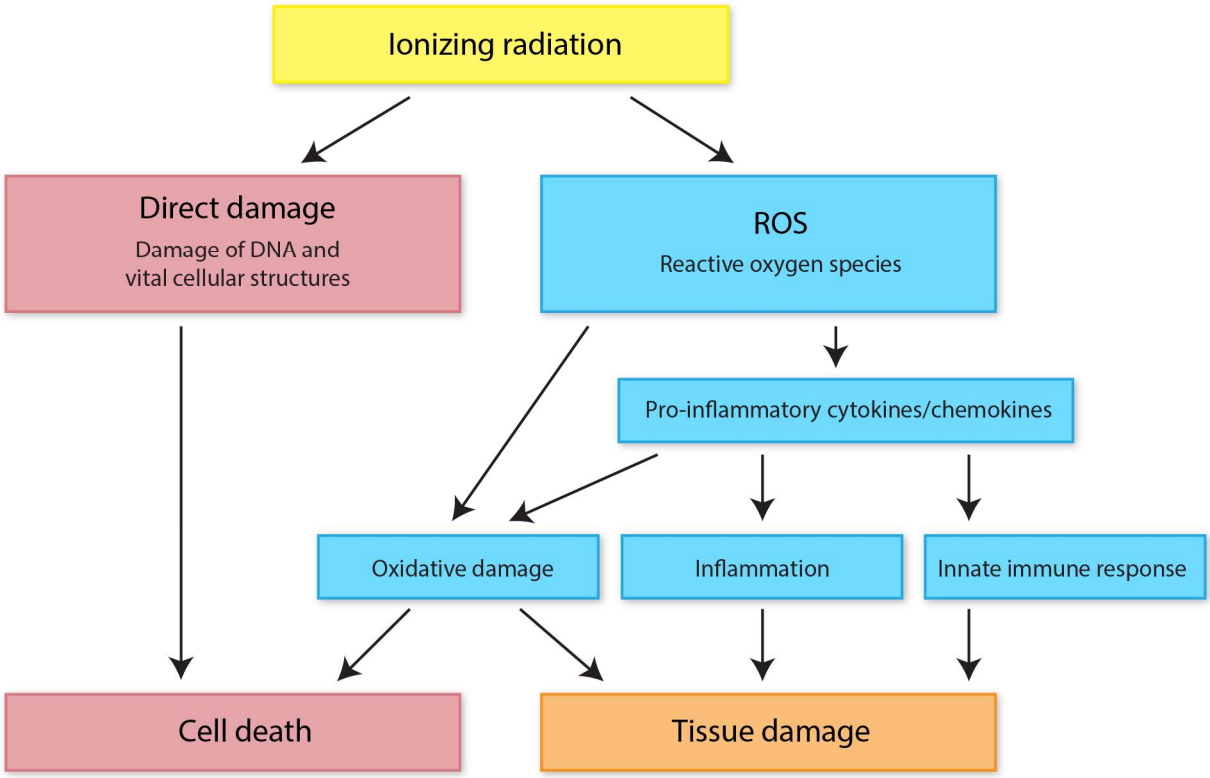

Figure 7. Irradiation can give rise to direct cellular death. In clinical doses, most cancer cells are killed via indirect effects mediated via ROS: ROS also initiates several effects in previously healthy cells, such as elevated oxidative stress and chronic inflammation.

1.4 RADIATION-INDUCED INJURIES

The risk of developing cancer increases with age; at the same time, life expectancy is increasing in most countries. Although the incidence rates of some forms of cancer are declining, in general, an increasing number of persons are being diagnosed with cancer. Consequently, a growing number of people will also undergo cancer treatment during their lifetime.[88] Fortunately, cancer treatments are becoming increasingly effective, with the five-year mortality rate falling to under 30% in wealthier parts of the world.[89] Paradoxically, with improved treatment for other diseases, such as cardiovascular diseases, cancer has become the leading cause of death in wealthy countries.[90]

At least 50% of cancer-treatment regimens include radiation therapy. Improvements in the administration of radiation therapy have led to a reduction in its adverse effects.[89] However, with a lower incidence of adverse effects, radiation doses have been increased for some forms of cancer in order to maximize the effect of the treatment.[91] Although the incidence of radiation-induced injuries is gradually decreasing, the prevalence of such injuries appears to have remained the same or may have even increased, since people are living longer, suffering from more types of cancer, and surviving for longer periods after their treatments.

The adverse effects of radiation therapy can be divided into acute and late, where the former is self-limiting, and the latter is chronic. Acute adverse effects may occur during radiation therapy and can be both local and systemic, causing symptoms from organs adjacent to the tumor and general symptoms, such as fatigue and nausea. These acute effects are mainly due to massive cellular death and subsequent reactions in the radiated area. When cells are killed, the body must break down and dispose of the residuals. This process is mainly carried out by different cells in the immune system and necrotic cells promote inflammation. Radiation therapy initiates an immune response that causes inflammation, the release of inflammatory cytokines, and the involvement of other systemically active agents that cause both local and systemic reactions.[92]

The most common manifestation of late radiation injuries are symptoms emerging or persisting for six months following radiation therapy.[93] The onset of late radiation-induced injuries has been reported to occur as late as 20 years from the radiation event, while the median time has been reported to be around three years.[94,95]

Many non-cancerogenic cells that are subjected to irradiation develop mutations in their DNA. These cells might be hindered from dividing, since their control systems detect damaged DNA and prevent them from entering the G0-stage.[96] These cells may remain in the resting stage for a long period of time, performing normal actions but never dividing. If a substantial number of cells in the affected

organ are unable to divide, then the density of healthy cells will decrease over time as an effect of aging. One cell line that is especially sensitive to this process is the endothelial cell line surrounding the blood vessels.[96,97] For this cell line, the blood supply becomes disrupted when a sufficient number of endothelial cells are depleted, which in turn leads to hypoxia in the tissue.[98] Hypovascularity, hypocellularity, and hypoxia are characteristic of radiation-induced injuries, a condition which Robert Marx referred to as the 3-H stage.[2]

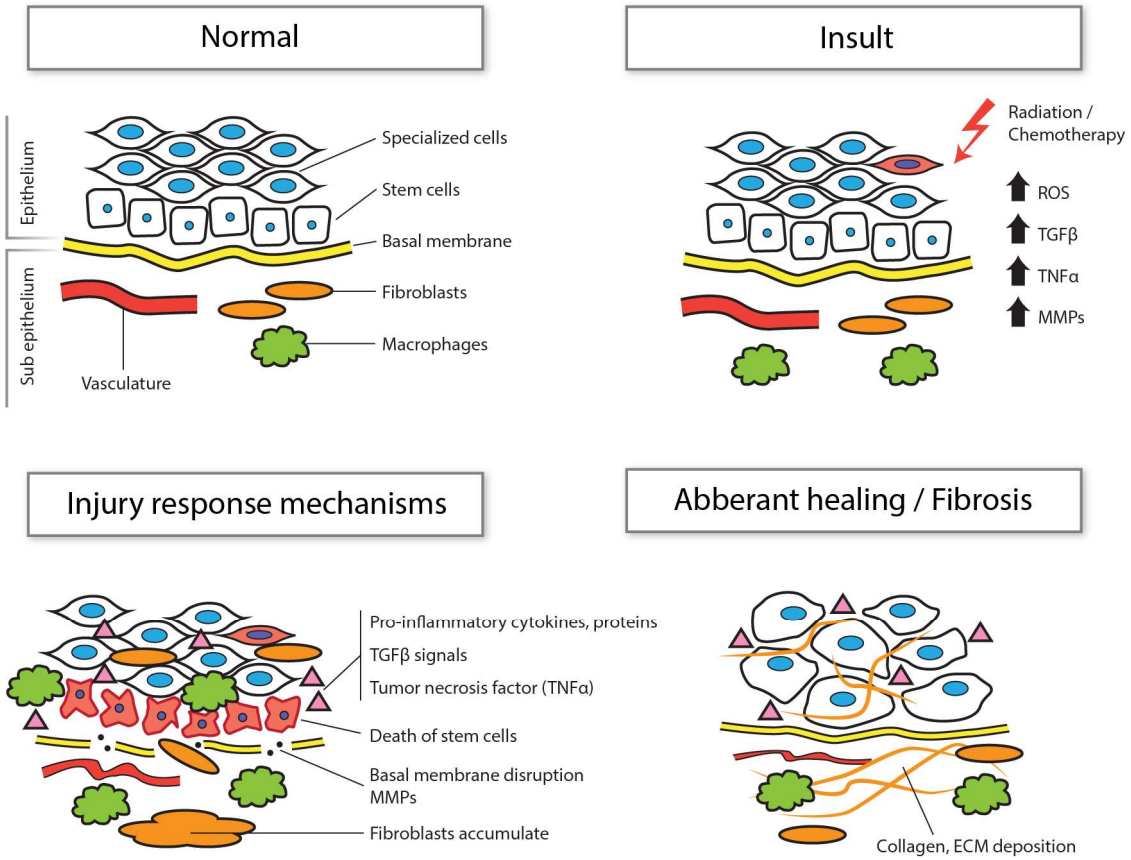

Figure 8. Irradiation initiates a vicious circle with chronic inflammation and fibrotic healing. The levels of ROS and fibrotic factors are elevated after irradiation. This leads to a disruption in the basal membrane and the infiltration of fibroblasts in the epithelium. Blood vessels are damaged, and collagen and the extracellular matrix are deposited in the epithelium.

There is a strong relationship between oxidative stress and chronic inflammation after radiation therapy.[99] The cellular response and death induced by radiation therapy leads to the recruitment of immunocompetent cells, such as macrophages and lymphocyte T cells. These cells release several inflammatory mediators, such as cytokines (IL-1, IL-2, IL-6, IL-8) as well as TNF, interferon gamma (IFN-γ), and TGF-β.[100] The release of these mediators initiates a secondary immunological response with the release of prostaglandins and free radicals, such as ROS,[101]which in turn leads to the recruitment of more inflammatory cells,

initiating a vicious circle.[99] This chronic inflammatory state leads to a malfunctional tissue repair process, culminating in the development of fibrosis, the depletion of organ-specific cells, and hypoxia (Figure 8).

Radiation is one of the few extrinsic activators of TBF–β, a cytokine involved in many cellular processes, such as cell proliferation and the production of the extracellular matrix.[102] TBF–β activates Smad proteins (transcription factors), and the increase of $Smad_3$ has been closely linked to the development of radiation-induced fibrosis.[103] TNF and IL-1 are pro-inflammatory and activate the secretion of matrix metalloproteinases (MMPs). MMPs are secreted as proenzymes that are activated by NO, oxygen and plasmin. MMPs can degrade the extracellular matrix and basal membranes, thus increasing fibrosis.[104]

The pathogeneses of adverse effects are similar in different organs, but the symptoms may differ depending on the organ affected. Fibrosis can cause impairment of vessel and parenchymal function since it restricts their function due to strangulation of all components within organs. This may result in the impingement of nerves or restrict passage through tubular organs, such as the urethra, trachea, intestines, or esophagus. Other organs, such as the urinary bladder, lungs, and heart, might have their volume or movement restricted due to fibrosis. The skin and other superficial tissues lose their elasticity, which can cause ulcers and impaired wound healing. If these ulcers occur in the intestines or urinary bladder, they can cause intermittent or chronic hemorrhage and fistulas.[105]

1.4.1 NORMAL FUNCTION OF THE URINARY BLADDER AND RECTUM

The main function of the urinary bladder and the rectum is to store urine and stool, respectively, and to allow their irregular and controlled release. Both organs are highly elastic, densely innervated, and well circulated. Distention as well as chemical stimulation initiates the urge to void urine or pass stool, an urge that can be suppressed until the autonomous nervous system overrides the voluntary signals to hold back.[106]

The epithelial lining of the urinary bladder is called the urothelium (Figure 9). It consists of three different levels of cells: basal, intermediate, and superficial. The basal cells situated just above the basal membrane are epithelial stem cells that provide long-term renewal of the epithelium. The intermediate cells are highly proliferative and can thus respond by quickly regenerating cells lost due to infection or injury. The superficial cells are fully differentiated and provide an impenetrable barrier for water, electrolytes, and other chemicals. All endothelial cells in these layers are connected to the basal membrane via filaments.[107]

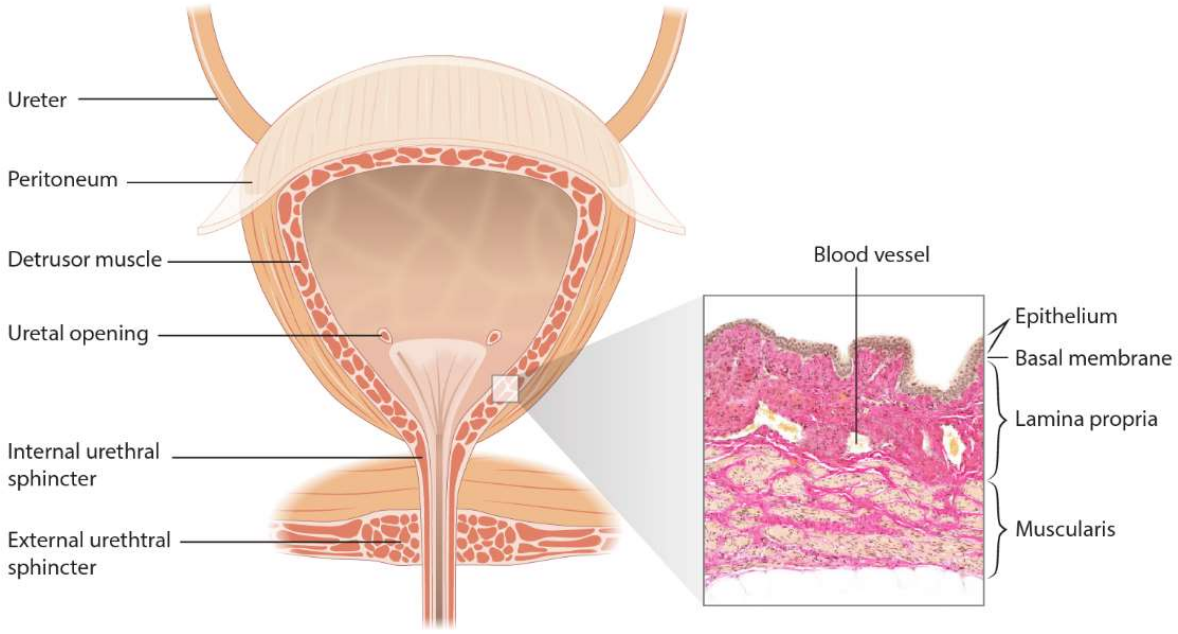

Figure 9. Normal anatomy and histology of the urinary bladder.

Underneath the basal membrane lies the lamina propria, followed by three different muscle layers. The lamina propria is filled with nerve endings, blood vessels, interstitial cells, fibroblasts, adipocytes, elastic fibers, and smooth muscle fascicles (muscularis mucosae). All layers are involved in the intricate signaling that regulates the distention of the urinary bladder and the urge to void. Disturbances in any of these layers or cell types might impair the overall function of the bladder, thus giving rise to an array of pathological conditions, such as radiation-induced injuries.[108]

The distal part of the sigmoid colon and rectum share several characteristics with the urinary bladder. Closest to the lumen is the intestinal epithelium or mucosa, with simple columnar enterocytes and goblet cells. Underneath the epithelium is the lamina propria, which is rich in blood vessels, lymph nodes, and loose connective tissue. The muscularis has one inner circular and one outer longitudinal musculature surrounded by the serosa.[106]

1.4.2 RADIATION-INDUCED INJURIES IN THE URINARY BLADDER

An increased proliferation of the urothelium and damage to the tight cellular junctions are seen following radiation therapy.[109] The normal polysaccharide layer is also damaged and, in combination, these effects lead to a pathologically increased permeability of the urothelium, allowing metabolites and bacteria to enter the underlying tissue.[93] This altered permeability is hypothesized to play a major role in the development of late radiation-induced injuries.[110,111] An increased

number of lysosomes and autophagic vacuoles are present in all cell layers, and multinucleate fibroblasts are seen together with edematous and necrotic endothelial cells.[112] When assessing the mucosa directly post-radiation, a diffuse mucosal edema followed by vascular telangiectasia, interstitial fibrosis, and mucosal bleeding is observed.[113]

As previously described, irradiation leads to increased oxidative stress in the tissue, demonstrated by elevated levels of 8–OHdG.[114] Downstream effects include elevation of TGF–β, IL–1, TNF, and ICAM–1, all related to the development of fibrosis, which restricts the function of the urinary bladder.[115] The regenerative process is impaired by the chronic inflammation, senescence, and malfunction of normal cells previously injured by irradiation. This may lead to malfunctioning revascularization with superficial and fragile blood vessels, which are prone to disruption and bleeding.[113] These changes may progressively deplete the blood supply to the urothelium, giving rise to chronic ischemia.[113]

1.4.3 PREVALENCE OF RADIATION-INDUCED INJURIES

Around 60% of male and 22% of female cancer survivors (on five-year follow-up) had previously been treated for cancer in the pelvic region.[116] Radiation therapy is given to around 50% of patients diagnosed with cancer, and around 40% of all cancers are cured with radiation therapy.[117]

The most common cancer in the male population is prostate cancer, with an incidence of around 200 per 100,000 male residents in Sweden (10,500 new cases per year).[116] This incidence increases with age and, with longer life expectancy, the incidence rate is expected to double by the year 2030.[118] Radiation therapy is given as the only treatment to one-third of patients and as an adjunctive to radical prostatectomy.[119] Treatment is effective, and "only" 25–20% of those diagnosed with prostate cancer will eventually die from the disease.[119,120] Approximately 50% of those treated for prostate cancer report bowel or urinary dysfunction to some degree.[121]

Other forms of cancer in the pelvic region, e.g., cervical, uterine, urinary, colon, and rectal cancer, are also treated with radiation therapy. Together, these cancer types constitute nearly 20% of all cancers.[116,119] Some of these cancer forms affect people of a younger age, with a long life expectancy after radiation treatment for, e.g., cervical and uterine cancer.[116] Hence, the time needed for late radiation-induced injuries to develop is longer, and those affected will therefore have to cope with their symptoms for longer.

Acute symptoms from the urinary bladder are reported by 23–80% of pelvic irradiated patients.[93] These symptoms usually subside within three to six months post radiation.[122] The risk of developing late radiation-induced injuries depends on an array of factors, such as the biologically active radiation dose, the organs

affected, and individual sensitivity.[123] The estimates of prevalence also depend on the diagnostic definitions used. Hence, the reported prevalence of more severe urinary and bowel dysfunction after radiation therapy varies greatly: 9–21% following treatment of prostate cancer, 3–7% for cervical cancer, and 2–47% for bladder cancer.[124] In one study 38% of women who had undergone radiation therapy due to cervical cancer reported chronic pelvic pain.[125] Stenosis and dyspareunia due to fibrosis is also common.[126,127] In one study, more than 30% were affected by bowel incontinence after radiation to the pelvic region.[128]

Patients suffering from late radiation-induced cystitis and proctitis are handled by doctors from different specialties, e.g., general practitioners, general surgeons, urologists, proctologists, or oncologists. This makes it difficult to obtain a complete overview of the incidence and prevalence rates of these conditions. The incidence rate of mild to moderate injuries may also be under-reported, since many patients are treated conservatively.

1.4.4 PATIENT-REPORTED SYMPTOMS

There is a large variation in the modality and severity of symptoms between individual patients. However, most patients experience a symptom-free interval that may last for months or several years post radiation.[123] The intensity of symptoms may vary greatly over time, but due to the progressive nature of the condition, these symptoms usually become more intense and persistent with time.[93,123]

Organ-specific symptoms of the late effects of radiation to the bladder and rectum can be summarized as:

- needing to pass urine/stool more often than usual (frequency)
- pain when passing urine/stool (pain)
- being unable to wait to empty the bladder/rectum (urgency)
- leaking urine/stool (incontinence)
- blood in the urine/stool (hematuria)
- difficulty passing urine/stool
- hard or loose stool
- stool in urine and/or vice versa (fistula)

These symptoms and limitations lead to reduced health-related quality of life.[129] Frequency, urgency, and incontinence all limit the patients' ability to take part in normal social activities and can often lead to frequent nocturnal disturbances.[129,130] Bleeding and fistulation can lead to formation of blood cloths, which may lead to urinary retention and anemia, requiring catheterization or urinary deviation, blood transfusion, and even cystectomy in the more severe cases.[131]

1.4.5 PATIENT-REPORTED OUTCOME MEASURES

Patient-perceived symptoms from the urinary bladder and rectum can vary in modality, frequency, and intensity and can affect the patient's life differently. A few different questionnaires have been used to assess late radiation-induced injuries in the urinary bladder and rectum.

- Late Effects Normal Tissue (LENT) Subjective, Objective, Management, Analytic (SOMA) scale
- Functional Assessment of Cancer Therapy for Prostate Patients (FACT-P)
- The Sexual Adjustments Questionnaire (SAQ)
- The American Urological Association (AUA) Questionnaire
- Expanded Prostate Index Composite (EPIC)

The LENT/SOMA scale exists for several organ systems and is widely used. It mixes patient-perceived symptoms, management, and objective findings. All aspects are scored from zero to four or as yes/no, and a total mean value of all aspects is calculated. It also requires a cystoscopy to be performed on each patient. The scale is not constructed for use with only one section, such as patient-perceived symptoms, and thus requires a full examination for complete scoring at each measuring point.[132]

The FACT-P, SAQ, and AUA questionnaires have been used as standalone questionnaires as well as in combination. The questionnaires are comprehensive but focus only on male sexual dysfunction, i.e., they are not applicable for female subjects.[133]

EPIC was originally constructed to evaluate symptoms in a male population. However, questions in two of the main domains are not gender-specific: urological and bowel. EPIC was later validated for use in a female population.[134] There are also sexual, hormonal, and health-related quality of life domains of EPIC. The different domains can be used separately from each other. There are two sections of each domain, one with qualitative questions and one with quantitative questions. Answers are given on a Likert scale, i.e., answers are converted to numbers between 0 and 100, and means are calculated for the whole domain or for sub-sections assessing specific symptoms (Figure 10).

The patient is instructed to consider an average during the past four weeks when answering EPIC. The quantitative questions in the urology domain are: How often have you leaked urine? How often have you urinated blood? How often have you had pain or burning with urination? Which of the following best describes your urinary control? (total to no control) How many adult diapers per day do you usually use to control leakage? The qualitative questions in the urology domain

are graded from “no problem” to “big problem”: How big problem, if any, has each of the following been for you? Dripping or leaking urine. Pain or burning on urination. Bleeding with urination. Weak urine stream or incomplete emptying. Waking up to urinate. Need to urinate frequently during the day. And finally: Overall, how big a problem has your urinary function been for you?

1.4.6 CLINICAL FINDINGS

Late radiation-induced cystitis and proctitis are both diagnoses of exclusion, meaning that other causes should be excluded before the patients can be diagnosed. Other reasons might be urinary infection, strictures, bladder stones, and cancer reoccurrence. Cystoscopy, an endoscopic examination of the urinary bladder, should be part of the examination. In more severe cases, telangiectasia, atrophy, erythematous mucosa, and bleeding may be seen during cystoscopy.[93]

Most patients with late radiation-induced injuries lack macroscopically evident changes. This was illustrated by a study in which 185 men with persistent hematuria, previously treated with brachytherapy for prostate cancer, underwent endoscopic evaluation. Of these patients, 9.6% were found to have a new bladder tumor. Although 70.8% of the patients in this study were diagnosed with late radiation-induced injuries, only 7% had macroscopic findings in support of the diagnosis at the endoscopic evaluation.[135]

1.4.7 CLINICAL OUTCOME MEASURES

Although objective findings vary and correlate poorly to patient-perceived symptoms, they are of interest in the assessment of the effects of HBOT in late radiation-induced injuries.

Specific classification scales are used for the clinical assessment of urinary- and bowel-related problems post radiation. The EORTC/RTOG classification is frequently used (Table 1).[136]

Bladder section of EORTC/RTOG

Findings/ Grade	0	1	2	3	4
Micturition schedule	None	None	Moderate frequency	Severe frequency and dysuria	Severe frequency and dysuria
Blood vessels	None	Minor telangiectasia	Generalized telangiectasia	Severe generalized telangiectasia (often with petechiae)	Severe generalized telangiectasia (ongoing bleeding)
Hematuria	None	Microscopic hematuria	Intermittent macroscopic hematuria	Frequent hematuria	Severe hemorrhage
Macroscopic epithelial appearance	Normal	Slight epithelial atrophy	Slight epithelial atrophy	Slight epithelial atrophy	Necrosis
Bladder capacity	>149 ml	>149 ml	>149 ml	<150 ml	<100 ml

*Table 1. EORTC/RTOG: The finding with the highest grade equals the score in the scale, i.e., it is **not** the mean value of all grades. Grade 5: Death directly related to late radiation effects.*

1.4.8 TREATMENT OPTIONS

Several reviews and guidelines exist that outline the best treatment options for radiation-induced cystitis and proctitis.[137] Mild cases are often treated conservatively with anticholinergic medications for frequency and urgency, analgesics for pain, and physiotherapy and incontinence pads for incontinence. While mild cases might be handled by general practitioners in primary care, more severe cases usually require specialist care. Radiation cystitis accounts for nearly 10% of admissions at some urology clinics.[138] Hospitalizations for radiation cystitis may be lengthy and costly.[139] There is also a lack of awareness among practicing urologist regarding available treatment options.[140]

Bladder irrigation with saline is usually the first-line treatment for hematuria and may be used to remove clots.[141] Agents such as alum are used for intravesical fulguration. These agents cause vasoconstriction and decrease capillary permeability, which may lead to reduced hematuria.[142,143] The efficacy and tolerability of intravesical alum has been reported in retrospective case series for several etiologies of intractable bladder hemorrhages.[144,145] Success rates vary but have been reported to be around 60%, yet nearly 70% of these “successes” ended in relapse within 1.5 years.[143]

Hyaluronic acid, a mucopolysaccharide, is believed to help restore the normal protective layer of polysaccharides that irradiation can damage.[146] Positive effects on bleeding as well as frequency, urgency, and pain have been reported.[147,148] Hyaluronic acid and hyperbaric oxygen had similar effects on hematuria in one study, with a response rate of 88–75%.[149] However, only 45–50% of the patients were free of symptoms at follow-up 18 months post treatment.[149]

There have been attempts to treat late radiation cystitis with other intravesical agents, such as antifibrinolytic agents, silver nitrate, and prostaglandins, but only in small studies and with uncertain results.[109] Systemically administered agents have also been tried, such as estrogen, macrophage regulators (WF10), and tranexamic acid, but none have proven to be effective.[109]

In the most severe cases, with bleedings requiring transfusions, transarterial embolization might be attempted. Cystectomy is the last resort and is associated with a high risk of morbidity and mortality. Severe complications are seen in one-third of the patients undergoing cystectomy, and the 90-day mortality rate is between 4.5–16%.[150,151]

1.5 HYPERBARIC OXYGEN

1.5.1 HYPERBARIC CHAMBERS

A hyperbaric chamber is needed in order to administer oxygen at a higher than normal ambient pressure. There are several different types of hyperbaric chambers, but for clinical purposes, they can be divided into mono- and multiplace chambers. The former has room for only one patient (or a parent and a child in some cases; Figure 11). The oxygen is normally administered without any breathing system, i.e., the whole chamber is flushed with oxygen. Multiplace chambers have room for two or more persons (Figure 10). The oxygen is delivered via a breathing system, e.g., a mask placed over the mouth and nose, or a transparent hood with a seal around the neck. The ambient gas in the multiplace chamber is normal air.[152]

Since monoplace chambers are flushed with oxygen, no additional respiratory dead space is created. In a multiplace chamber, where masks or hoods are used, additional dead space is created. In addition, ambient air can leak in through the seal of the mask or hood and further dilute the inspired oxygen. In combination, these effects can contribute to making the inspired partial pressure of oxygen lower for patients treated in multiplace chambers even when the ambient pressure is the same.[153] This fact explains why monoplace treatment tables often utilize a lower total pressure compared to their multiplace counterparts, e.g., 200 kPa and 240 kPa, respectively.[152]

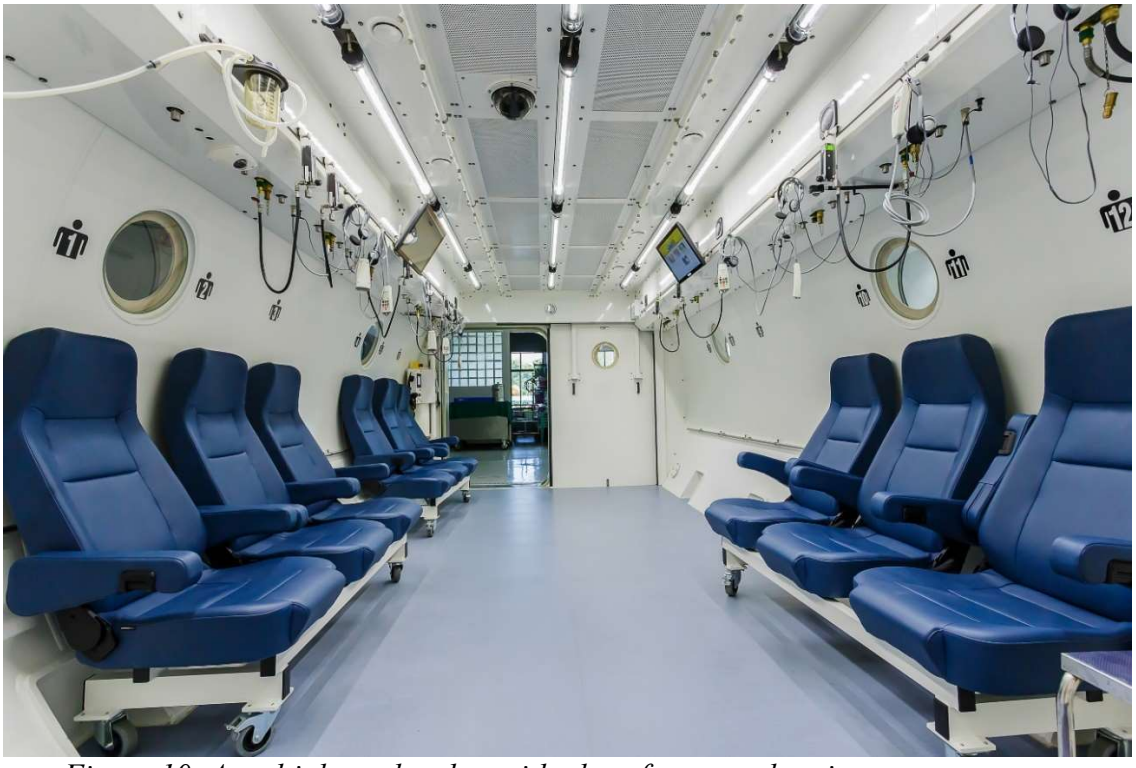

Figure 10. A multiplace chamber with place for several patients.
Image copyright Kamolrat | Dreamstime.com, Image ID: 41315236

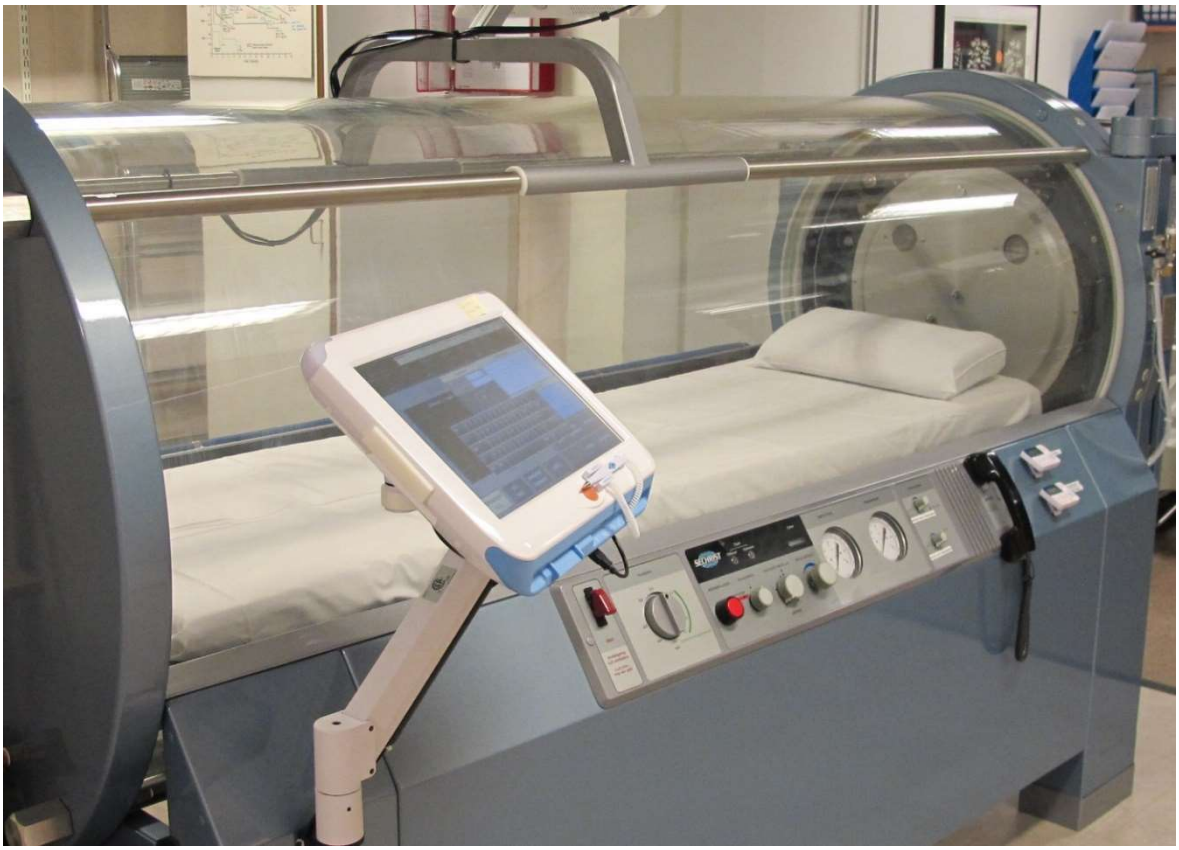

Figure 11. A monoplace chamber for one patient.
Image Courtesy of Joakim Trogen, Sahlgrenska University Hospital/Östra, Göteborg, Sweden.

Depending on the purpose and use of the hyperbaric chamber, it can be equipped with inlets for additional breathing gases and advanced monitoring devices. Modern multiplace chambers can usually accommodate patients requiring intensive care, e.g., when treating carbon monoxide poisoning or necrotizing fasciitis. Many hyperbaric chambers in hospitals are designed for a maximum absolute pressure of around 400 kPa. Hyperbaric chambers designed for offshore and diving activities can be pressurized to much higher absolute pressures.[152]

1.5.2 EFFECTS OF HBOT

Several different actions of HBOT have been observed and investigated. These actions, either alone or in combination, contribute to the total effect of the treatment for the respective indication. Hyperoxia leads to increased oxidative stress and supernormal levels of ROS.[81] This is a key mechanism through which HBOT exerts some of its effects.[154]

1.5.3 PHYSICAL EFFECTS

Compression: Boyle's law predicts that the volume of a gas is reduced when the pressure is elevated.[155] HBOT will thus compress gas bubbles in the body and stop the disruption of blood flow, which may be blocked by gas emboli in smaller

vessels. This effect is desirable when treating decompression sickness and arterial gas embolism.[152]

Diffusion and elimination: Henry's law predicts that gases will dissolve in liquids to an extent determined by the equilibrium between the undissolved gas and the dissolved gas in the liquid. The diffusion gradient, i.e., the difference in the partial pressure of a gas, between the blood and alveoli affects the rate of gas exchange. Inhalation of pure oxygen at a high partial pressure leads to a steep diffusion gradient for nitrogen, which accelerates its elimination compared to simply breathing air. Nitrogen is the gas responsible for bubble formation in decompression sickness and arterial gas emboli.[48]

Diffusion and distance: The distance to which a gas can diffuse from a capillary depends on the partial pressure gradient of the gas between the blood vessel and the tissue. The diffusion distance is increased with elevated partial pressures of oxygen. In cases where the blood flow is partially disrupted, e.g., diabetic foot ulcers, this effect can be utilized to oxygenate hypoxic tissues that would otherwise become necrotic.[152]

Osmosis: The steep gradient of oxygen concentration between plasma and tissues creates an osmotic effect. The molecular diffusion of oxygen from plasma to tissue is coupled with a diffusion of nitrogen in the opposite direction. Together, these effects create a net flow of extracellular fluids from tissues to blood vessels, thus creating an anti-edema effect.[156] In tissues in which hypoxia has already caused increased permeability of the blood vessels and other cellular responses that contribute to edema, the restoration of oxygenation may also contribute to an anti-edema effect.[157]

1.5.4 BIOCHEMISTRY

Since oxygen is involved in an abundance of microchemical processes, increased oxygen levels have the potential to affect a multitude of these processes.

Vasoconstriction: Arteries possess potassium-dependent channels that are activated during hypoxia. This leads to hyperpolarization and relaxation of vascular smooth muscle cells, which in turn causes vasodilation and increased blood flow.[158] Hyperoxia counteracts this mechanism, and vasoconstriction is further enhanced by the direct effect on L-type Ca^{2+} channels via angiotensin II and the potent vasoconstrictor 20-Hydroxyeicosatetraenoic acid.[157]

Reperfusion: Disruption of blood flow leads to hypoxia and subsequent damage to cells and tissues, eventually culminating in necrosis and cellular death. This process can be reversed if the blood flow is restored, but some of the inflammatory processes initiated by hypoxia contribute to additional tissue damage.[159] Reactions initiated at reperfusion involve the formation of cytotoxic oxidants derived from

molecular oxygen. The polymorphonuclear leukocyte is a major source of reactive oxygen metabolites in post-ischemic tissues. Neutrophils are the primary mediators of the reperfusion-induced increased permeability of blood vessels.[159] An adhesion molecule, β_2 integrin, is responsible for persistent adherence of neutrophils to the endothelium.[160] If HBOT is administered *before* hypoxia, then the expression of β_2 integrin is suppressed and the potential for reperfusion injury is significantly diminished.[161-163]

Inflammation: Inflammation can be induced, modulated, and terminated via several different pathways depending on the underlying cause.[164] Indeed, the resolution of inflammation is an active and tightly regulated process.[165] Hypoxia plays an important role in inflammation.[166] The inflammatory response seen in tissues after trauma is characterized by the release of cytokines, the activation of neutrophils, and enhanced microvascular adherence.[167] HBOT suppresses inflammatory cytokines, such as TNF production, augments prostaglandin E_2 (PGE_2), cyclooxygenase–2 (COX–2), and IFNγ release, and increases the anti-inflammatory cytokine IL-10.[168-170]

Angiogenesis: As previously explained, hypoxia in a cell leads to increased levels of ROS and increased levels of nitric oxide. One of the downstream effects is the increased production of angiogenetic factors, such as HIF–1α and VEGF.[171,172]

The elevated production of HIF–1α and VEGF is induced by HBOT through the upregulation of specific genes, i.e., *c-Jun, ERK, JNK,* and *AP–1*, via ROS and nitric oxide activation.[173]

Endothelial progenitor cells (EPCs) play a central role in angiogenesis. HBOT contributes to a significant increase of EPCs released from the bone marrow that are required to repair the hypoxic area.[174] Other angiogenetic factors, such as angiopoietin, are also upregulated by HBOT via ROS-dependent pathways.[175]

1.5.5 EFFECTS ON HOST INFECTION RESPONSE

Hypoxia generally favors infection and impairs endogenous response.[176] Infection contributes directly to hypoxia since microbes consume oxygen. Hypoxia is further aggravated by the increased demand from surrounding tissues and infectious response cells.[177] Cells involved in infection defense, e.g., neutrophils, polymorphonuclear cells (PMNs), and macrophages, all utilize more oxygen. Neutrophils and PMNs produce ROS and superoxides and release them in the vicinity of bacteria in order to kill them.[178] This ability is highly impaired when tissue oxygen levels drop below 4-5 kPa.[176,179,180] Macrophage function is also highly impaired by low oxygen tension. Hypoxia may trigger the release of TNF, IL-1, and IL-8, which can adversely affect the infection response.[181]

HBOT increases tissue oxygen levels far above the lower threshold needed for neutrophils and PMNs to function optimally. Indeed, the function of PMNs reaches supranormal levels during hyperbaric oxygen conditions and kills bacteria more efficiently.[182,183] The function of macrophages is also restored during HBOT.[184]

Some antibiotic agents, such as gentamicin and tobramycin, are unable to penetrate bacteria if the oxygen tension is low. Others, such as ciprofloxacin and aminoglycoside, have oxygen-dependent killing effects on bacteria. The effects of these antibiotics are enhanced with elevated partial pressures of oxygen in the tissue.[179,185,186]

1.5.6 EFFECTS ON BACTERIA

Anaerobic bacteria are highly susceptible to high partial pressures of oxygen.[187] One reason for this is that such bacteria have low or no levels of SOD and are hence highly vulnerable to ROS.[188] Oxygen acts bacteriostatically on anaerobic bacteria at 30 kPa and becomes bacteriocidic for strict anaerobes at oxygen partial pressures above 60 kPa.[184]

Some bacteria, such as Clostridium perfringens, release alpha toxins that cause tissue injury and cellular death. High partial pressures of oxygen via HBOT suppress the production of these toxins and thus reduce their harmful effects. [180,189-191]

1.5.7 WOUND HEALING

Several different processes are involved in wound healing and recovery from tissue damage. Hypoxia is normal in the acute phase of injury and triggers some important hemostatic processes, but chronic hypoxia impairs wound healing.[192] Chronic hypoxia due to microvascular changes, and fibrosis are seen in conditions like diabetes mellitus and after radiation therapy.

Collagen is an important factor in wound healing. The production and development of collagen are directly correlated with the partial pressure of oxygen. The function of several enzymes involved in cross-linking collagen and thus stabilizing the wound are related to the partial pressure of oxygen.[193,194] The production of collagen is proportional to the partial pressure of oxygen up to 15 kPa and may be further enhanced by HBOT.[194-196] The differentiation of keratinocytes and the proliferation of fibroblasts, also involved in the stabilization of healing tissue and the epithelialization of the wound, are increased by HBOT.[197]

Angiogenesis is important in wound healing and, as previously mentioned, HBOT mimics the hypoxic stress response by elevating ROS and nitric oxide with subsequent effects on key mediators for angiogenesis, i.e., HIF-1α and VEGF.[198]

1.5.8 HBOT IN THE CLINICAL SETTING

While any elevation of the ambient pressure and content of oxygen in the inspired air will meet the definition of hyperbaric oxygen, it is only over certain levels that clinical effects are observed. Undersea & Hyperbaric Medicine Society (UHMS) defines HBOT as treatment with 100% oxygen at an absolute pressure exceeding 140 kPa.[152]As with all other pharmaceutical agents, the bioactive dose and the duration of exposure are highly relevant factors when assessing the response.

As previously mentioned, HBOT has been tested on an array of different conditions at different pressures, for varying durations and numbers of sessions. Preclinical studies have shown the effects of HBOT on cells, tissue, organs, and microbes alike, and clinical studies have shown measurable and relevant effects in some conditions. However, many studies are of poor quality, and HBOT is sometimes used for conditions for which scientific proof of effect is lacking or highly questionable, such as autism, cerebral palsy, and multiple sclerosis.[4-6]

Oxygen can be considered a pharmaceutical drug, and some countries restrict its use in medical conditions, but it is also readily available without prescription and can be used without medical supervision, e.g., when scuba diving. Hence, non-medical or para-medical entities can market and deliver HBOT for all sorts of conditions. This fact highlights the importance of separating scientifically proven indications from unscientific results.

1.5.9 INDICATIONS FOR HBOT

There are several international scientific communities devoted to hyperbaric medicine that have assessed available knowledge and published lists of "approved" indications for HBOT. The largest community, UHMS, is based in the US and issues such a list in collaboration with the Food and Drug Administration (FDA). The European Underwater and Baromedical Society (EUBS) and the Swedish Society for Anesthesiology and Intensive Care Medicine (SFAI) both have scientific reference groups for hyperbaric medicine. SFAI issues a list of "approved" indications for HBOT in Sweden—yet for some indications, the interpretation of scientific evidence differs, and hence the lists are not identical.

SFAI states that HBOT can be considered for the following 10 conditions:[199]

- Decompression sickness
- Gas embolism
- Carbon monoxide poisoning and fire/smoke intoxication
- Diabetic foot ulcers
- Late soft tissue radiation injury
- Osteoradionecrosis

- Severe acute tissue ischemia
- Necrotizing soft tissue infections
- Intracranial abscess
- Osteomyelitis

1.5.10 ADMINISTRATION OF HYPERBARIC OXYGEN

Time and pressure can be used to express the dose of oxygen delivered. However, it is important to understand that the pressure needs to be sufficiently high in order to achieve some of the desirable effects of HBOT. Longer expoure times at lower pressures will not compensate for this, even if the amount of oxygen delivered is the same. Hence, time and pressure must be assessed separately and in combination when assessing the dose of oxygen delivered.

Some of the effects of HBOT are only seen when the partial pressure of oxygen exceeds a certain level. Boerema used 300 kPa when treating gas gangrene, but at this partial pressure of oxygen, toxicity is a problem.[26,200] At the same time, many of the desirable effects described above are not seen at oxygen partial pressures below 160–200 kPa.[37,182,195,201-204] Marx showed that HBOT, with oxygen partial pressures over 200 kPa, induces an upregulation of angiogenetic factors in previously irradiated bone, leading to increased vascular density, whereas normobaric oxygen (100 kPa) does not.[37]

In order to compensate for the additional dead space and potential leakage when masks and hoods are used, Davis' original pressurization tables for hyperbaric oxygen treatment in multiplace chambers used 236 kPa.[200] (Actually, Davis originally chose 250 kPa, but since the pressure gauge was measured in feet of seawater (fsw), and the corresponding value (49.5 fsw) was hard to read, Davis backed down to 45 fsw, which corresponds to 236 kPa. Again, this is often rounded off to 240 kPa in chambers using pressure gauges with kPa or Bar).[205]

The duration of treatment was also derived from the original work of Boerema on gas gangrene and was set to 90 minutes by Davis.[26,200] Air brakes, in which the administration of oxygen is interrupted for 5–10 minutes, were introduced to further lower the risk of oxygen toxicity.

For elective treatments, for which angiogenesis is one of the desired effects, Marx showed macroscopic signs of new blood vessels after 24 HBOT sessions.[37] Later work has often used 30 or 40 HBOT sessions in an attempt to maximize the effect for these indications.[206,207] However, dose-response studies in a clinical setting are lacking. Individual susceptibility and response to HBOT might vary, and this should be considered when deciding on when to terminate the treatment. One complicating factor is that there is a delay for some of the desired effects of HBOT to be clinically evident, e.g., size reduction of visible wounds and the alleviation

of symptoms.[208] In the absence of reliable and relatively early markers of effect, hyperbaric clinics around the world tend to use a standardized number of treatment sessions for elective indications, usually between 30 to 40 treatments.

The desired effects are different for acute indications, for which the treatment pressure and duration as well as the number of HBOT sessions will differ from those given electively. Decompression sickness is often treated at higher pressures (280 kPa) in order to maximize the physiological compression effect on the bubbles and for a longer duration (5 to 6 hours). Carbon monoxide poisoning is normally treated with one HBOT session, whereas necrotizing fasciitis usually requires several sessions during a short period of time.[152]

1.5.11 ADVERSE EFFECTS OF AND CONTRAINDICATIONS TO HBOT

HBOT is regarded as a safe and well-tolerated treatment with few contraindications.[152,209] Middle-ear barotrauma, with varying degrees of bleeding or membrane ruptures, can occur if the patient fails to equalize pressure. While this injury is often mild and heals without scars, it is also preventable by guidance and instructions to the patient or by a small incision of the eardrum, i.e., myringotomy.[152] Myopia, with changed vision can occur after repeated HBOT.[210] The condition is partly due to the hardening of the lens, which leads to increased refraction.[211] However, the condition is reversible for the vast majority of patients.[210]

Oxygen-induced seizures are extremely rare (< 1/1000 treatments) in normal clinical settings with the use of modern treatment tables. Such seizures are self-terminating if the oxygen level is reduced, and patients typically recover fully from the event.[152]

An absolute contraindication for HBOT is unventilated pneumothorax due to the risk of developing tension pneumothorax. Patients with pulmonary disease, such as chronic obstructive pulmonary disease or untreated asthma, can have unvented pulmonary sections (bullae) that can rupture and cause a pneumothorax during HBOT. Severe congestive cardiac failure can be aggravated by HBOT due to increased vascular resistance and increased workload for the heart. These conditions are relative contraindications for HBOT.[152]

Severe claustrophobia might make patients reluctant to accept HBOT. Acculturation, sometimes with the addition of mild sedation with benzodiazepines, is usually enough to overcome this hurdle.[152]

1.5.12 NEW AND RECURRING CANCER AFTER HBOT

The levels of oxygen and ROS are increased during HBOT. Theoretically, this could lead to the development of new cancer cells or benefit already existing tumors. However, the level of oxidative stress induced by HBOT alone has not been linked to an increased frequency of new cancers or to the stimulation of tumor growth, nor has it been shown to promote cancer recurrence.[32,212]

1.6 CLINICAL STUDIES ON HBOT AND LATE RADIATION-INDUCED INJURIES

Late radiation-induced injuries and their treatment with HBOT have been of research interest for several decades, and several papers have reported favorable outcomes. Traditionally, soft and bony tissues are separated when assessing the clinical outcome of treatment in this condition. Although there are differences between these two types of tissue, their similarities in terms of the genesis of injuries as well as the effects of HBOT are apparent. For practical reasons, most studies focus on one organ, tissue type, or cell line, which might make it difficult to draw conclusions about their clinical implications.

A health technology assessment (HTA) was made prior to the first study (Paper I) in order to establish the current scientific support for HBOT.[213] Only controlled studies were considered when grading the scientific proof in this HTA report. Radiation-induced injuries in the urinary bladder (cystitis) were separated from those in the lower part of the intestine (proctitis) in this assessment. We searched PubMed from Jan 1, 1970 to April 5, 2011 for "cystitis" OR "proctitis" AND "radiation" OR "radiation injuries" [Mesh] AND "hyperbaric" OR "hyperbaric oxygenation" [Mesh] OR "HBO" [tiab] OR "HBOT" [tiab], limiting our results to the English, German, Danish, Norwegian, and Swedish languages. There was only one controlled, but non-randomized, study for cystitis, and one controlled and randomized study on proctitis. The conclusion was that there is some support for HBOT in radiation-induced soft tissue injuries, but there is also a clear need for more clinical studies.[213] In a more recent systematic review, published by the Cochrane Library by Bennet et al., one of the main conclusions was as follows: *"These small trials suggest that for people with LRTI [red. late radiation tissue injury] affecting tissues of the head, neck, anus and rectum, HBOT is associated with improved outcome."*[137]

Listed in the HTA report, but not part of the final assessment of effect, are several uncontrolled and retrospective clinical trials that reported positive effects of HBOT for cystitis and proctitis. A positive response rate of 70–90% was reported in all but two of the papers.[206,214-224] In the less-positive report on hemorrhagic cystitis, three out of eleven patients had a positive response to HBOT.[225] One paper on proctitis reported a response rate of around 50%.[226] Only a few studies have assessed symptoms other than hemorrhage, but these studies reported alleviation of frequency, urgency, incontinence, and pain from the urinary and bowel, as well as positive effects on health-related quality of life (HRQoL).[206,220,227,228] One study found that early treatment, defined as the treatment of hematuria within six months of onset, gave a better response rate than late treatment: 27 of 28 (96%) patients treated early had partial or complete resolution of hematuria, while only 21 of 32 (65%) patients treated late had similar response respectively.[219] One of two

randomized, controlled, and blinded studies assessed the effects of HBOT for proctitis. It showed a significant reduction of symptoms and clinical findings measured with SOMA-LENT: 12.55 to 7.48 and 12.82 to 10.23, respectively; (p=.0019) with the number needed to treat for resolution estimated to be 3.[227] The other randomized, controlled trial, published in 2015, showed no effect of HBOT when applied for chronic bowel dysfunction after pelvic radiotherapy.[229] However, this study has been widely criticized for selection bias, employed a flawed evaluation method, was missing data, and was shown to have been underpowered.[230-236]

In conclusion, a few high-quality studies have reported a positive outcome of HBOT for radiation-induced soft tissue injuries. Several smaller studies have also supported the use of HBOT for these conditions. Several preclinical papers have assessed the underlying mechanisms and effects that HBOT may have. The available knowledge lends support to several causative explanations for how HBOT can reverse the effects induced by radiation therapy. Many questions remain, however, and thus there is a need for additional studies in the field.

2 AIMS

Paper I:

Assess whether HBOT could reduce patient-perceived symptoms of late radiation-induced cystitis and proctitis.

Paper II:

Assess whether radiation-induced oxidative stress reaction and subsequent inflammatory and pro-fibrotic response in the urinary bladders of rats could be reversed by HBOT, as well as whether HBOT alone triggered any response in the studied parameters.

Paper III:

Establish a cellular model for the irradiation of urothelial and endothelial cells, with subsequent exposure to HBOT, by assessing cell proliferation at varying irradiation doses and protocols for HBOT.

Paper IV:

Assess whether HBOT could alleviate patient-reported symptoms of late radiation-induced cystitis and reduce or reverse injuries in the urinary bladder in a randomized, controlled, multicenter trial. A secondary aim was to assess whether HBOT affected HRQoL.

3 METHODS

The papers in this thesis range from a prospective, longitudinal cohort study (Paper I) via an animal model (Paper II) and a cell model (Paper III) to a clinical, multicenter, randomized, controlled study (Paper IV). A detailed description of the methods used can be found in the Methods section of the respective papers.

3.1 ETHICS AND APPROVALS

Studies involving humans (Papers I and IV) were conducted in compliance with the International Council for Harmonization of Technical Requirements and the ethical principles of the Declaration of Helsinki. These studies were approved by the Ethics Review Board in Gothenburg, Sweden (Dnr 025-10: T108-12 and T213-13). Paper IV was approved by the National Medical Product Agency of Sweden and registered in the European Union Drug Regulating Authorities Clinical Trials Database and in ClinicalTrials.gov. All patients were fully informed about their participation in the respective trials and gave their written and oral consent. Both papers were prepared in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

The study involving animals (Paper II) was performed according to the National Institutes of Health guidelines for the care and use of laboratory animals and was approved by the Animal Ethics Committee at the University of Gothenburg (157-2013). The paper was prepared in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

3.2 STUDY-SPECIFIC METHODS

3.2.1 PAPER I

Subjects: All patients referred for HBOT with the diagnosis of late radiation-induced cystitis or proctitis were invited to participate in the study. There were no additional inclusion or exclusion criteria other than the general contraindications to HBOT. Patients were recruited during a four-year period (Jan 2008 to Dec 2011), and a total of 39 patients gave their consent for inclusion.

Design: This was a prospective cohort study with an evaluation of patient-perceived symptoms before, directly after, and six to twelve months post HBOT. A two-year follow-up with EPIC was also part of the design.

Data collection: EPIC was used for the evaluation of patient-perceived symptoms. Patients were asked to fill in the EPIC questionnaire before the start of HBOT, directly after the last HBOT session, and again six to twelve months later. Demographic data and medical histories were collected retrospectively using a predefined form.

Procedures: Patients were treated with 100% oxygen in either a multiplace chamber at an ambient pressure of 240 kPa or in a monoplace chamber at 200 kPa. Time at pressure was 90 minutes, and the treatment was given once daily, five days weekly. The patients initially received 30 sessions of HBOT for fewer than 45 days before assessment for clinical response was made. Patients were categorized into four groups: healed, improved, unchanged, and worse, where "improved" patients were offered an additional 10 sessions of HBOT within two months of the end of the first sessions. This treatment protocol was used in the HORTIS-III study published in 2008 and was judged to be the best way to titrate the total HBOT dose.[227]

Statistical analysis: The EPIC scores obtained before, during, and six to twelve months after HBOT were assessed for variance and analyzed using Tukey's post hoc test. The mean EPIC score was compared for each specific subset of EPIC questions using a paired parametric 2-tailed t test.

3.2.2 PAPER II

Subjects: 39 female Sprague-Dawley rats.

Design: Rats were divided into four groups, with one group serving as the control and thus receiving no study-specific treatment, one group receiving radiation only, one group receiving HBOT only, and the last group receiving both radiation and, later, HBOT.

Data collection: Analysis of mRNA was performed using q-PCR with TaqMan gene expression assays. Immunohistochemistry was done on paraffin-embedded 6µm-sections. Fluorescence was measured at three representative areas and converted to percentages of the maximum value. The assessor was blinded to group allocation.

Procedures: Radiation was given as one fraction of 20 Gy at day 1. At day 15, HBOT was given at 200 kPa, with 100% oxygen, for 90 minutes, bi-daily for 10 days, totaling 20 sessions. All animals were sacrificed on day 29. The urinary bladder was cut into two sagittal parts, with one put in formalin for later paraffin embedding, and the other frozen for mRNA analysis.

Statistical analysis: Analysis of variance (ANOVA) was used to determine significant differences between mean values. Tukey's HSD post-hoc analysis was used for multiple comparisons between groups.

3.2.3 PAPER III

Cells: Human-immortalized UROtsa and HUVEC cell lines.

Design: Cells were exposed to radiation in incremental doses (0– 20 Gy). Cell death and ED50 were assessed 24 hours later. Cells were then divided into four groups: no intervention (control), irradiation ED50 dose (radiation), HBOT (HBOT), and ED50 followed by HBOT (radiation + HBOT). Additionally, HUVEC cells were given two HBOT sessions at different time points after radiation.

Data collection: The number of cells were normalized to the control, which was set to 100%. Number of cells were counted manually in four representative fields and the mean value was used for comparison between groups.

Procedures: Cells were grown in flasks using a specific cell medium until cellular confluence was reached, which was defined as 100% cells in each vision field. One group of cells was used as the control. Two groups were irradiated using a R 2000 x-ray irradiator at the aforementioned doses. One of the irradiated groups was exposed to HBOT at different time intervals. One group was only exposed to

HBOT. HBOT was delivered at 200 kPa using 100% oxygen for 90 minutes per session.

Statistical analysis: Mann-Whitney's test was used for comparison between groups and ANOVA to test for significance.

3.2.4 PAPER IV

Subjects: All patients between 18 and 80 years of age with late radiation-induced cystitis as their reason for referral were invited to participate in the study. Time from radiation to inclusion had to be over six months. Patients with very mild symptoms (EPIC total urinary score >80) were not included, nor were patients who had ongoing bleeding requiring blood transfusion (> 0.5L the last four weeks). All patients underwent cystoscopy before randomization, and patients were excluded if other reasons for the symptoms were found or if their urinary bladder capacity was under 100 ml. Also, patients requiring urinary deviation, such as urinary or pigtail catheters, were excluded due to the design of EPIC.

Design: Data included patient medical history, EPIC, SF-36, clinical assessment, and cystoscopy with biopsies. All data were entered into an electronic case report form (e-CRF), where 1:1 randomization in blocks of four was done automatically. Stratification was done for gender (male or female), time from radiation to inclusion (less or more than 2 years), and previous intrusive surgery in the pelvic region (yes or no). Patients randomized to intervention underwent HBOT, while the control group continued with the previous treatment and did not receive any study-specific treatment.

The evaluation of the treatment effect was done with EPIC and SF-36 directly after the end of the HBOT protocol for the intervention group. A safety panel of blood samples were also taken directly after the end of the HBOT sessions. The primary endpoint was assessed six to eight months post randomization during a visit that was identical to the screening visit. The results in the intervention group were compared with the results in the control group. Safety data (adverse events) were collected during HBOT.

Patients in the control group were offered HBOT after the assessment of the primary endpoint. All patients will be followed for five years with yearly assessment of EPIC and SF-36 (Figure 12).

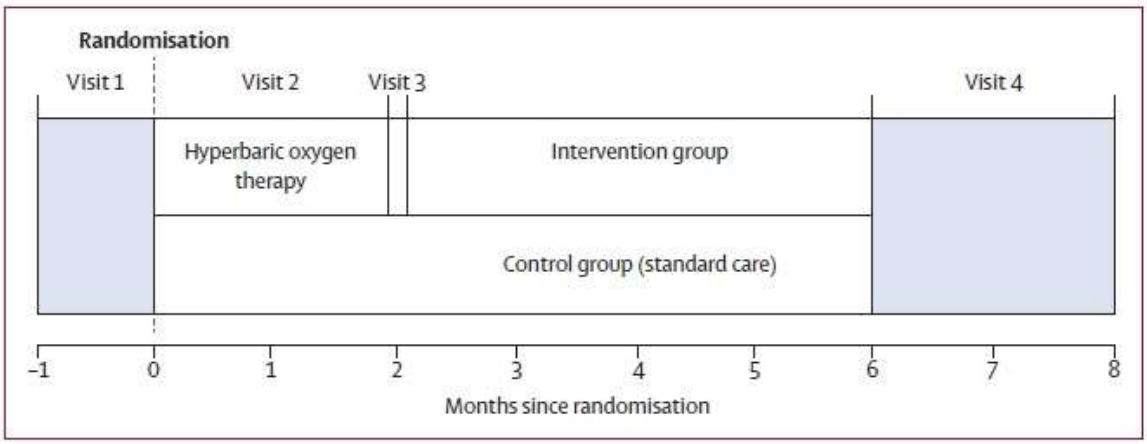

Figure 12. Randomization took place directly after screening (visit 1). HBOT was given to the intervention group (visit 2). A safety visit (visit 3) was done after HBOT. Visit 4 was done 6–8 months after randomization (i.e., 4–6 months after completion of HBOT). Visit 1 and visit 4 were identical for the two groups.

Data collection: All data collection was performed in the e-CRF with automatic contingency checks and validation. Investigators and patients were asked to complete specific forms in accordance with the protocol. The study was monitored with the verification of source data, time restrictions, signatures, management, approvals, and other critical parameters.

Procedures: Patients were treated with 100% oxygen in either a multiplace chamber at an ambient pressure of 240 kPa or in a monoplace chamber at 200 kPa. Time at pressure was 90 minutes and was given once daily, five days weekly, during 30–40 sessions.

Cystoscopy was performed by study-specific urologists according to a standardized protocol. Biopsies were taken on all patients except those whom the urologist judged to be at high risk of bleeding or other complications. The findings were recorded in the e-CRF and automatically transformed to a score on the urinary bladder RTOG/EROTC scale.

Statistical analysis: Sample size was calculated using the data from the pilot study (Paper I) and was set to 40 in each group. All data were signed and locked before finishing the study. The statistical analysis plan was written and signed before any export of data from the e-CRF-system. The difference in EPIC urinary total scores between the groups was tested for normality and skewness with Q–Q plots and kurtosis, and via statistical inference with Student's two-sample t test.

4 RESULTS

The main results from each paper are summarized in this section. The results are presented in full in the respective papers found in the appendix.

4.1 PAPER I

During the study period (Jan 2018 to Dec 2011), 52 patients were referred for late radiation-induced cystitis or proctitis. Of these, 39 patients completed HBOT and had valid EPIC forms for evaluation (Figure 13).

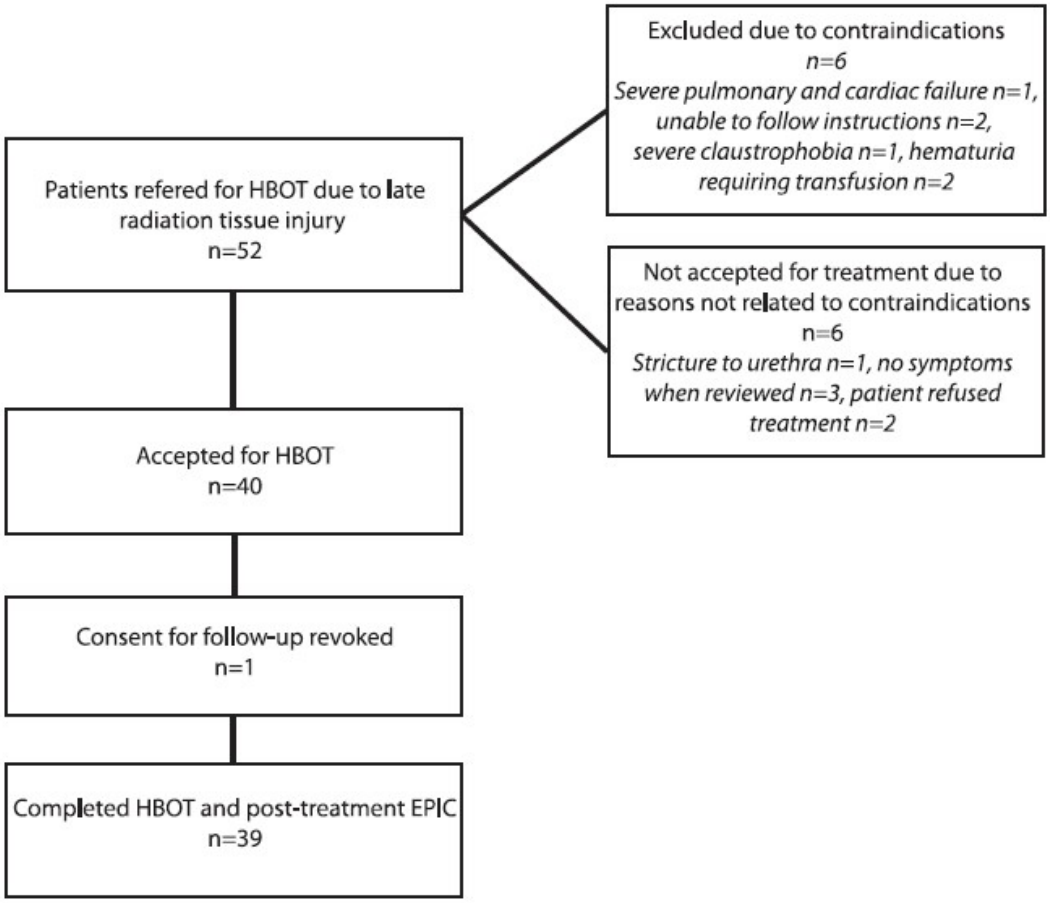

Figure 13. Of 52 patients reviewed for inclusion, 12 were not included, leaving 40 eligible patients. After one dropout, 39 patients remained part of the evaluation.

Patient demographics and medical history	
Number of patients	39
Age (mean)	71 (35–84)
Sex	
Female	4
Male	35
Tumor and treatment	
Prostate	34
External radiation (70-75 Gy)	23
Brachy therapy (28-33 Gy)	3
External (50 Gy) and brachy (20 Gy)	8
Radical prostatectomy	13
Rectal	3
External radiation (25 Gy)	1
External (50 Gy) + chemotherapy	2
Rectal resection	3
Cervix	2
External radiation (60-64 Gy)	2
Hysterectomy	1

Table 2. Demographic and cancer treatment medical history.

Demographic and medical histories are reported in Table 2. The mean time from the end of radiation therapy to urinary symptoms was 18 (range 0–120) months, while the mean time from the end of radiation therapy to bowel symptoms was 5.5 (range 0–120) months. Twenty patients (51%) were affected in terms of both their urinary and bowel function when assessed at baseline. Nine patients (23%) had only urinary symptoms, and seven patients (18%) had only bowel symptoms. The remaining three patients (8%) had EPIC scores over 80 in both domains. The mean number of HBOT sessions was 36 (range 28–40).

For all patients, regardless of symptoms, the total EPIC score at baseline was 60.9±22.6 (n=38) for urinary and 62.2±26.5 (n=38) for bowel. The scores increased to 74.0±21.0 (n=37; p<.0001) urinary and 76.2±20.4 (n=36; p<.0001) for bowel directly after completion of the HBOT session, and remained stable at follow-up six to twelve months later: 73.4±23.6 (n=36; p=0.0002) for urinary and 73.3±20.2 (n=36 p=0.0001) for bowel.

For patients with urinary symptoms, defined as EPIC < 80 in the urinary domain, the total urinary EPIC score at baseline was 50.3±15.5 (n=29). The score increased to 65.5±18.9 (n=27; p=0.0002) directly after completion of the HBOT sessions, and further to 68.6±20.1 (n=27; p<.0001) compared with baseline at follow-up six to twelve months later (Figure 14).

For patients with bowel symptoms, defined as EPIC < 80 in the bowel domain, the total bowel EPIC score at baseline was 48.1±17.6 (n=25). The score increased to 67.5±17.9 (n=25; p<.0001) directly after completion of the HBOT sessions and was 66.5±19.1 (n=25; p<.0001) compared with baseline at follow-up six to twelve months later (Figure 14).

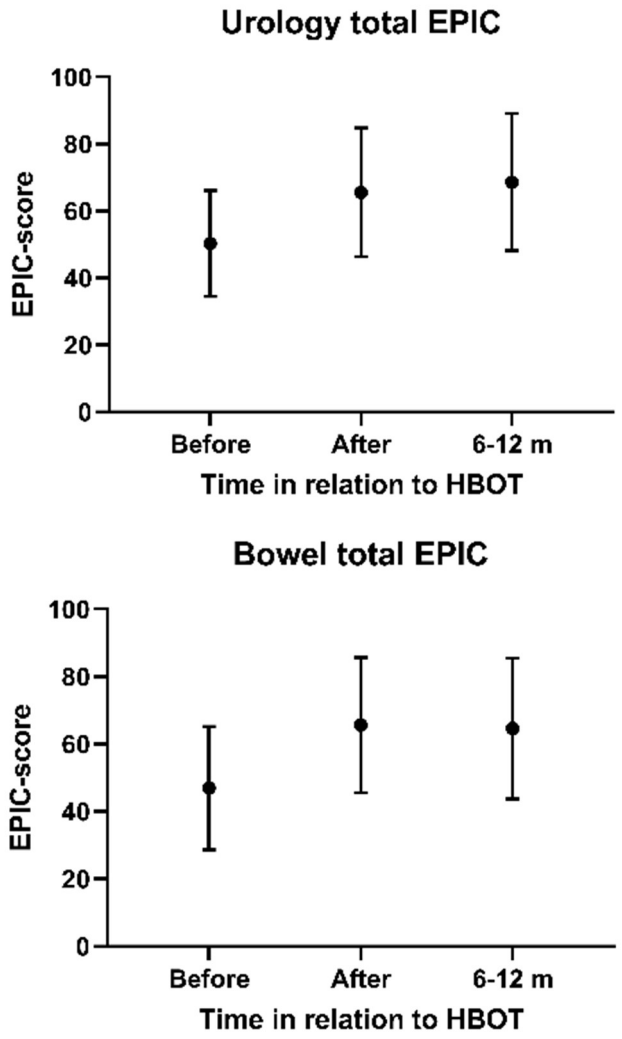

Figure 14. Total bowel and urinary EPIC scores before, directly after, and at follow-up 6–12 months after HBOT for all patients with symptoms in the respective domain.

4.2 PAPER II

The rats exposed to irradiation had an upregulation of oxidative stress marker 8-OHdG with a mean intensity value of 34±14% (n=4) compared with 5±2% in the control group (n=4; p<.0001). NRF2α, SOD–2, HO-1, IL-10, and TNF were also upregulated by irradiation, (n=7‑10; p<0.001‑0.05) as shown in Figure 15, while TGF–β (n=7; p=0.63) and SOD–2 (n=7; p=0.58) were not.

Rats exposed to irradiation and later to HBOT were compared with irradiated rats (no HBOT). 8-OHdG was restored to 11±5% (n=4; p<.0001), as were all other expressions elevated by radiation alone (n=7-10; p<.0001-0.05) (Figure 15).

The rats only exposed to HBOT exhibited no significant changes compared to rats in the control group (Figure 15). The additional inflammatory parameters assessed, i.e., IL–1, IL–4, IL–5, IL–6, IL–13, and INF–γ were not affected in any group.

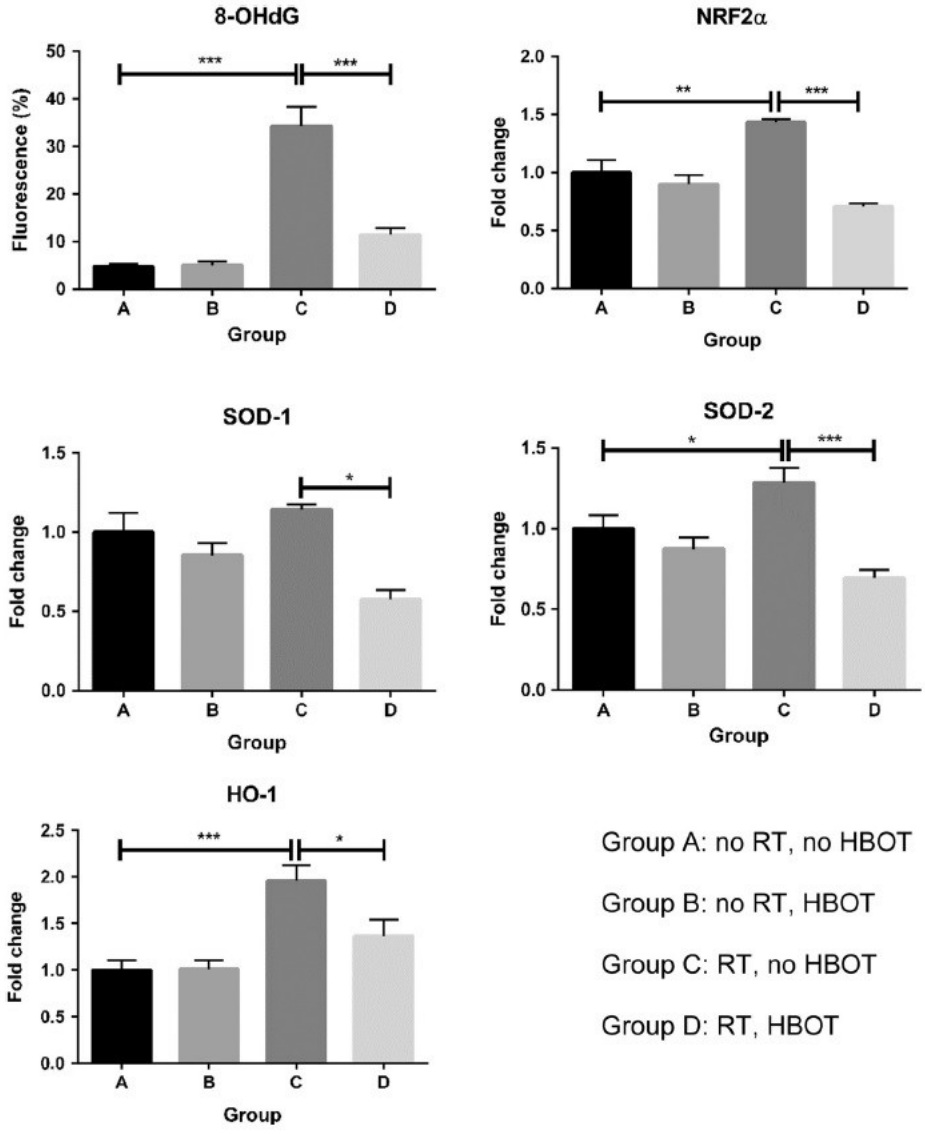

Figure 15.Continued on next page

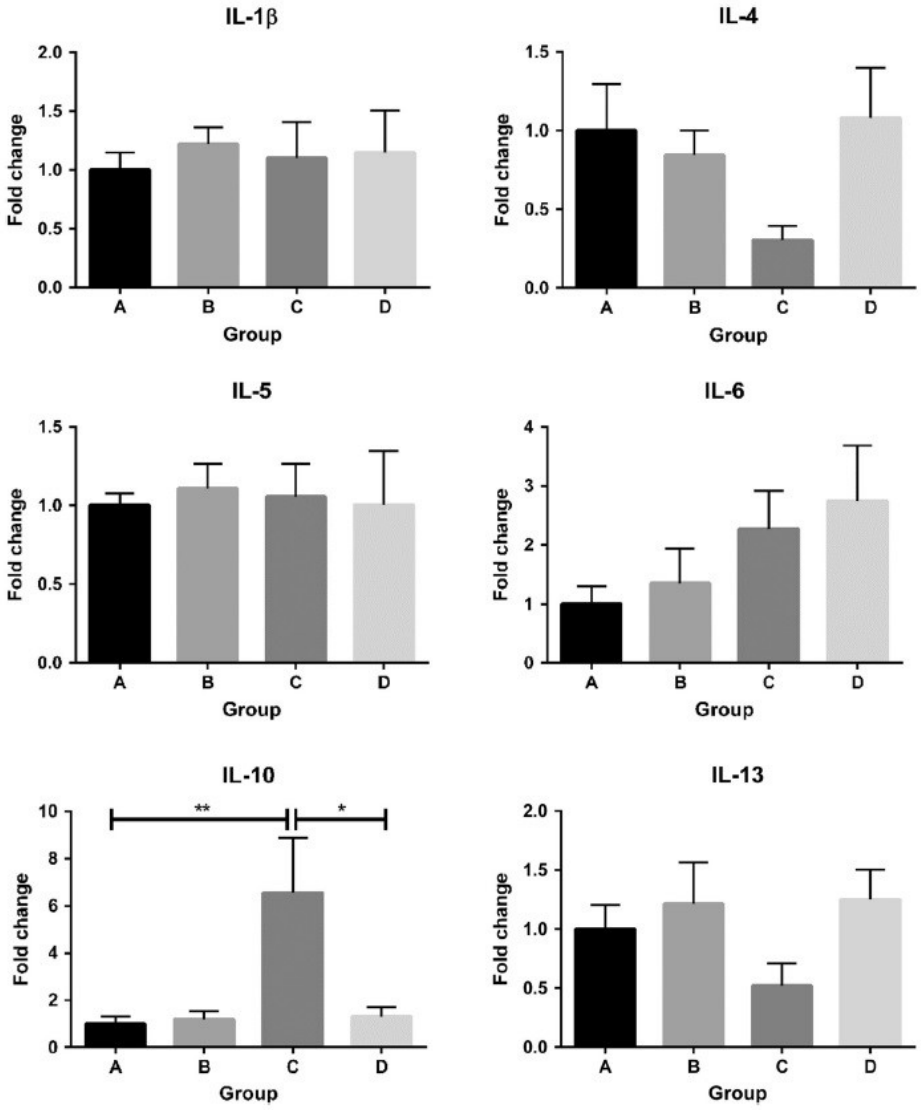

*Figure 15. Urinary bladder expressions of mRNAs in controls (no RT, no HBOT), HBOT control group (no RT, HBOT), irradiated group (RT, no HBOT), and HBOT irradiated group (RT, HBOT), respectively (n=8–10). * indicates p<0.05 and ** indicates p<0.01. Vertical bars represent SEM.*

Immuno-histochemical analyses showed signs of increased oxidative stress predominately in the urothelium. An increase in oxidative stress was seen not only in the nucleus but also in the cytoplasm. SOD–2, seen in the urothelium and blood vessels, and OH–1, seen in the blood vessels, were increased after radiation and restored after HBOT (Figure 16).

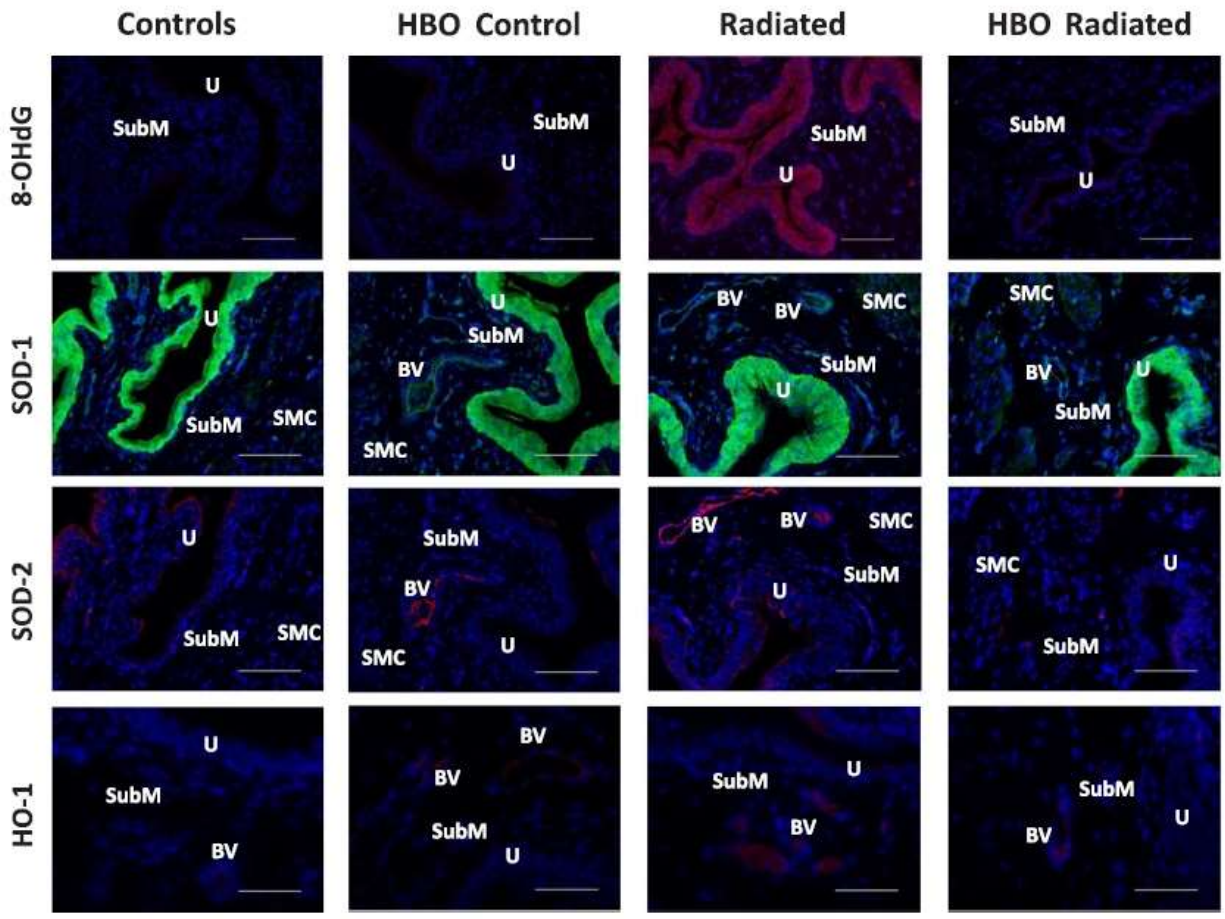

Figure 16. Photographs of urinary bladder specimens stained for 8-OHdG, SOD-1, SOD-2, and HO-1 in the respective groups. Upregulation of 8-OHdG was observed in the urothelium in the radiation group, which was reversed by HBOT (first row). SOD-2 was primarily upregulated in blood vessels and in the urothelium by bladder irradiation (third row). BV=blood vessel(s), SMC=smooth muscle cells, SubM=submucosa, and U=urothelium. Horizontal bars indicate 100 µm.

4.3 PAPER III

Irradiation induced cellular death when assessed 24 hours after irradiation. Effective dose 50% (ED50) was reached at 6 Gy (Figure 17).

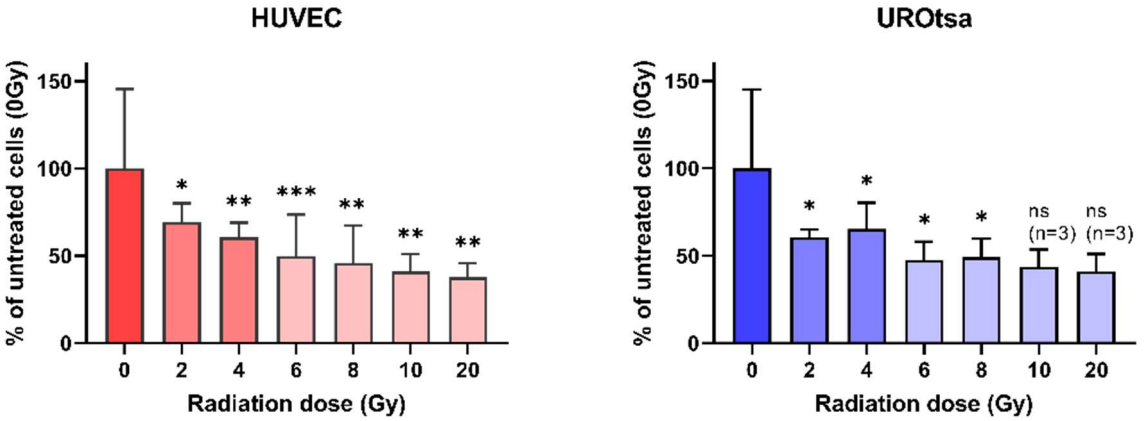

*Figure 17. H Number of cells normalized to control (0 Gy). Number of wells counted per radiation dose: HUVEC n=7 UROtsa n=4-3. Standard deviation is indicated with whiskers, * = p<0.05, ** = p<0.01, *** = p<0.001, ns = no significance.*

Radiation caused a significant decrease in proliferation (100% to 67%, n=8, p=<0.0001). Irradiation followed by one session of HBOT restored the cell numbers to higher than initial values (128%, n=8, p=<0.0001) HUVEC cells exposed to one HBOT session after irradiation did not recover from cellular death induced by 6 Gy (Figure 18).

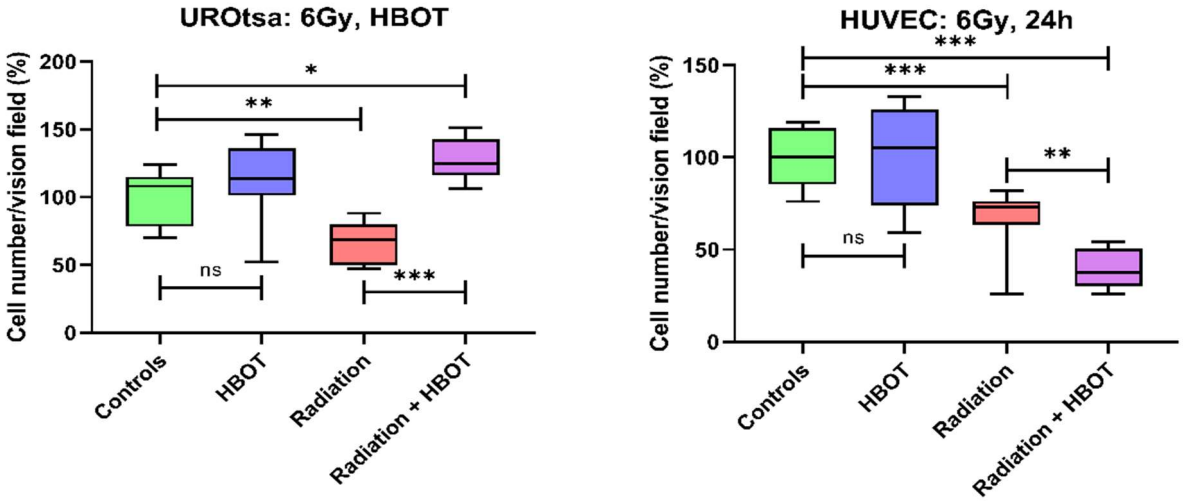

*Figure 18. Number of cells are normalized to control (0 Gy). Standard deviation is included in the box, with the whiskers indicating a 95% confidence interval, and the bar showing the median value, * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001, ns = no significance.*

Two sessions of HBOT given at three and six hours after radiation also decreased cell numbers compared to only radiation. However, two sessions of HBOT given at three and ten hours after irradiation led to an increase in cell numbers. The same pattern was observed when the cells were assessed both 24 and 48 hours after radiation (Figure 19).

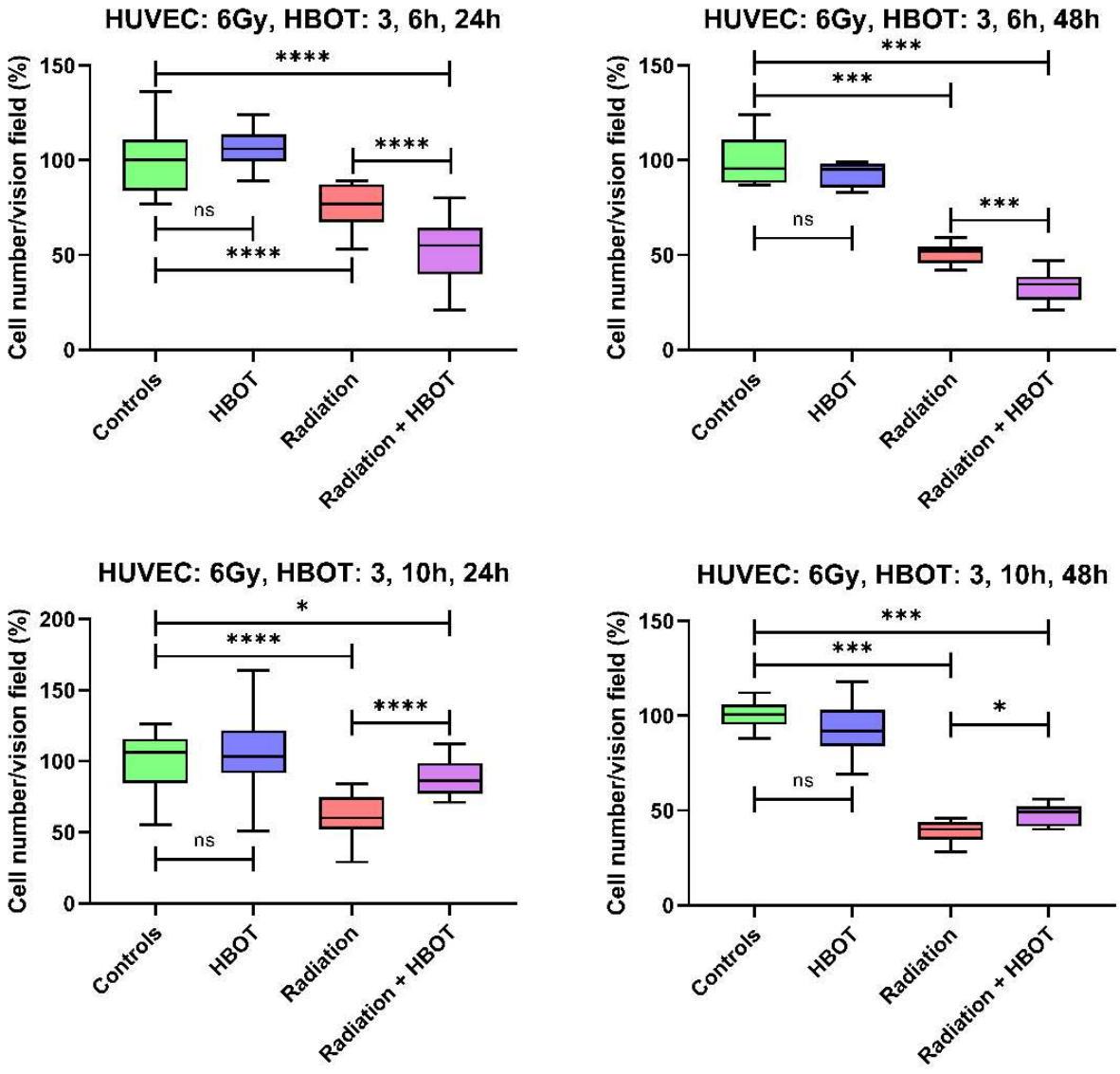

*Figure 19. Number of cells are normalized to control (0 Gy). Standard deviation is included in the box, with the whiskers indicating a 95% confidence interval, and the bar showing the median value, * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001, ns = no significance.*

4.4 PAPER IV

The study included 87 of 223 patients screened between May 9, 2012 and December 20, 2017. Of the patients, 42 were randomized to HBOT, and 45 received standard care. Eight patients withdrew consent directly after randomization and were thus excluded from the intention-to-treat (ITT) population: one patient in the HBOT group, and seven patients in the standard care group. Hence, 79 patients were included in the ITT analyses. Group means and individual changes in EPIC scores are shown in Figure 20.

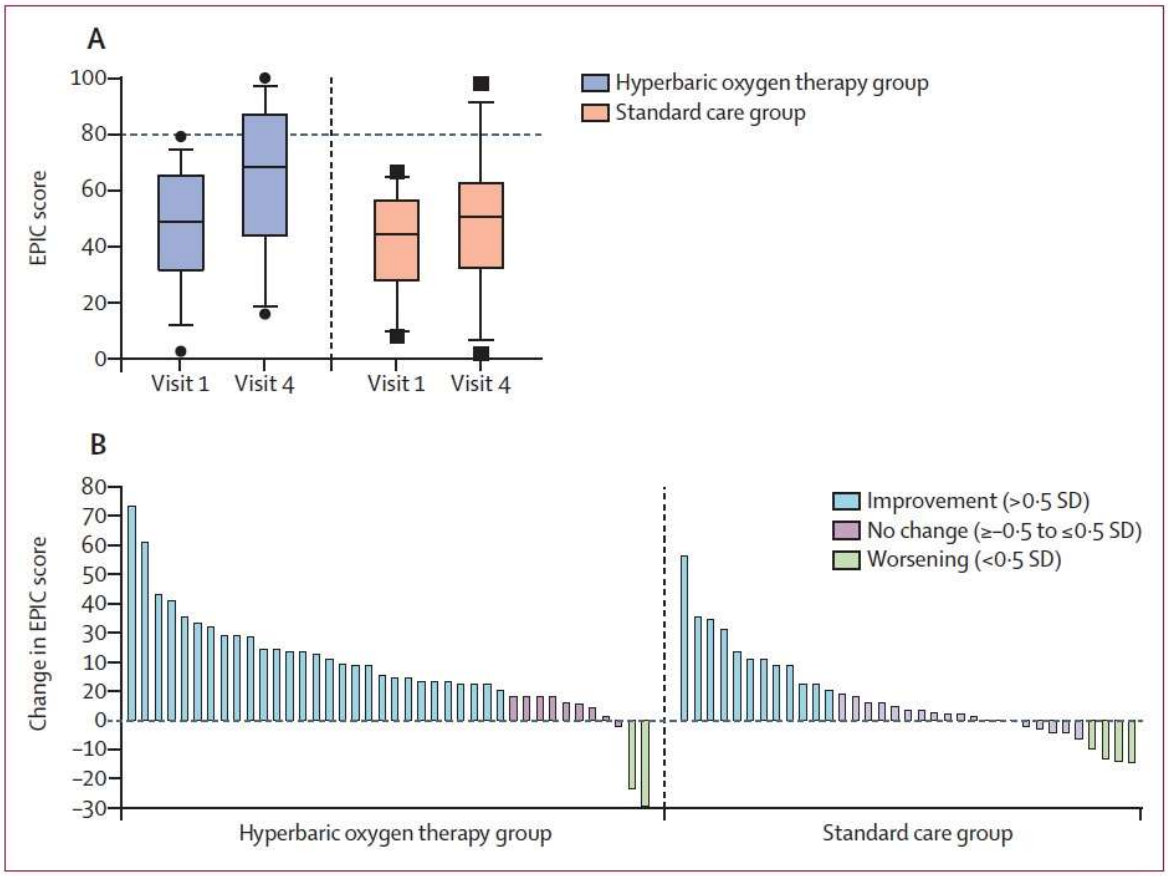

Figure 20. Intention-to-treat population. (A) Changes in EPIC scores between visit 1 and visit 4. The group mean is shown with a line, and the whiskers represent the 5th-95th percentiles. Outliers are marked with a solid circle or square. The dotted line at a score of 80 indicates the cut-off for inclusion. (B) Individual changes in EPIC scores between visit 1 and visit 4. The patient in the standard care group who improved by more than 40 points unintentionally received HBOT before evaluation at visit 4.

Four patients from the standard care group were excluded before visit 4. One was lost to follow-up, one was diagnosed with a new cancer, one died, and one patient was unintentionally treated with HBOT despite his group allocation. One patient in the HBOT group discontinued due to an adverse event. The per-protocol population thus included 34 patients in the standard care group and 40 patients in the HBOT group.

In the per-protocol population, the difference between changes in the group mean of the EPIC urinary total score at visit 4 was 11.4 points (95% CI; 3.5–19.2; p=0.005; 17.8 points [SD 18.4] in the HBOT group vs. 6.6 points [13.2] in the standard care group) (Table 3).

Visit 1 Baseline	HBOT (n=40)	p-value within group	Standard care (n=34)	p-value within group	p-value between groups	Difference between group mean (95% CI)
Urological Total	48·0 (19·2)		42·0 (18·0)		0·11	
Urological Function	56·0 (23·3)		48·5 (23·6)		0·18	
Urological Bother	42·3 (19·9)		37·4 (17·1)		0·27	
Urological Incontinence	49·0 (32·9)		36·6 (28·1)		0·091	
Urological Irr./Obstr.	49·8 (20·0)		46·4 (18·9)		0·47	
Bowel Total	60·6 (21·2)		61·9 (23·1)		0·81	
Visit 4	HBOT (n=40)	p-value within group	Standard care (n=34)	p-value within group	p-value between groups	Difference between group mean (95% CI)
Urological Total	65·5 (24·6)		49·0 (24·5)		0·0056	-10·0 (-18·2 to -1·8)
Urological Function	69·1 (28·8)		52·9 (27·3)		0·018	
Urological Bother	62·9 (24·6)		46·2 (24·2)		0·0056	
Urological Incontinence	60·4 (36·7)		36·9 (29·9)		0·0047	
Urological Irr./Obstr.	69·2 (22·7)		56·0 (25·0)		0·021	
Bowel Total	73·5 (16·4)		67·3 (23·5)		0·19	
Change from Visit 1 to Visit 4	HBOT (n=40)	p-value within group	Standard care (n=34)	p-value within group	p-value between groups	Difference between group mean (95% CI)
Urological Total	17·8 (18·4)	<0·0001	6·6 (13·2)	0·0049	0·0047	-11·4 (-19·2; -3·5)
Urological Function	13·8 (19·8)	<0·0001	4·7 (14·4)	0·070	0·032	-9·1 (-17·3; -0·84)
Urological Bother	20·7 (20·1)	<0·0001	8·0 (14·6)	0·0031	0·0035	-12·7 (-21·0; -4·3)
Urological Incontinence	12·8 (18·5)	<0·0001	0·76 (15·3)	0·78	0·0041	-12·0 (-20·1; -3·9)
Urological Irr./Obstr.	19·6 (21·8)	<0·0001	9·0 (14·9)	0·0014	0·022	-10·6 (-19·4; -1·6)
Bowel Total	13·2 (17·3)	<0·0001	4·1 (12·7)	0·075	0·017	-9·0 (-16·3; -1·7)

Table 3. Per-protocol analyses: EPIC scores at visit 1 and visit 4, presented as means (SD). Differences between group means are presented together with a 95% confidence interval (95% CI).

The number of patients who scored 80 or higher on EPIC at visit 4 was 16 (40%) of 40 in the HBOT group, and two (6%) of 34 in the standard care group.

Mean SF-36 scores increased significantly within the HBOT group from visit 1 to visit 4 in four of the eight domains. Compared to the control group, the HBOT group improved significantly in general health (p=0.0002) and physical functioning (p=0.038).

The HBOT group had higher LRMGS grades than in the standard care group at visit 1, but the difference was not significant (p=0·068). At visit 4, 64% of the patients in the HBOT group had improved grades, 28% had unchanged grades,

and 8% had worsened grades. In the standard care group, 18% of the patients had improved grades, 53% had unchanged grades, and 29% had worsened grades (p=0.0012). Figure 21 depicts the intention-to-treat population.

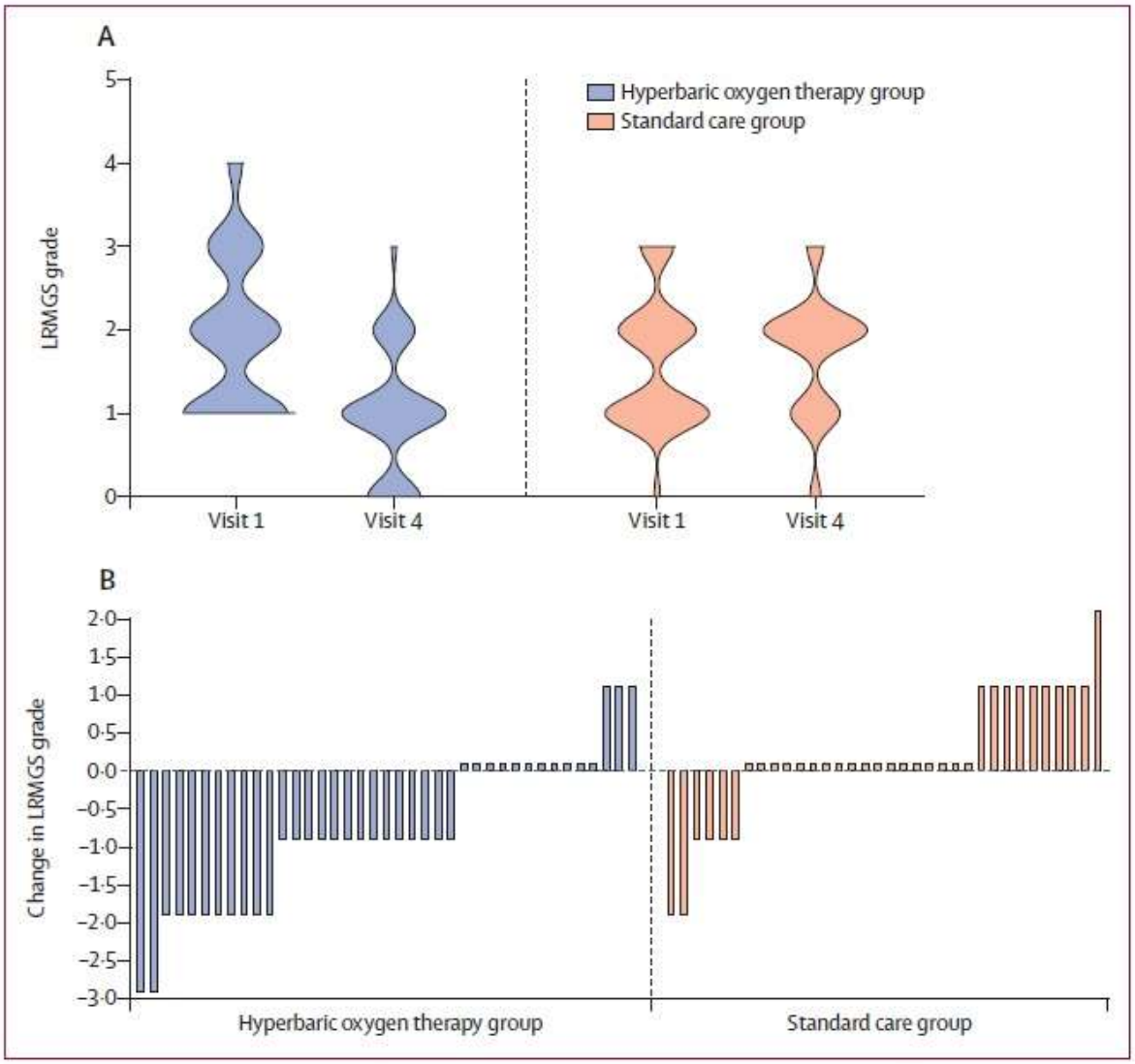

Figure 21. Intention-to-treat population: (A) Changes in LRMGS grades between visit 1 and visit 4, where a lower score equals improvement and HBOT leads to a significant reduction compared to the control (p=0.0012). (B) Individual changes in LRMGS grades between visit 1 and visit 4. A lower LRMGS grade means fewer pathological findings.

All patients in the HBOT group complied with the predefined protocols, and no dose adjustments were required. We recorded 43 adverse events affecting 17 (41%) of 41 patients in the HBOT group: difficulty equalizing pressure in the middle ear (n=9), signs of barotrauma (n=4), paracentesis (n=1), transient myopia (n=5), Leber's hereditary optic neuropathy (n=1; discontinued HBOT due to visual disturbances), and panic attack (n=1). The rest of the adverse effects were not classified as related to HBOT, including one serious adverse event (death due to sepsis and cardiac failure).

5 DISCUSSION

This chapter is chronologically structured to reflect the scientific process discussed. The reasons for initiating the respective studies are explained along with the knowledge considered in their design. Also, the findings for the respective studies are related to previous findings and placed in context. Several questions were answered, but many new questions have emerged.

5.1.1 DOES HBOT HELP AT ALL? – PAPER I

Knowledge regarding HBOT for late radiation-induced injuries was assessed in an HTA report in 2012.[213] HBOT seemed to be effective, but the evidence was weak. For proctitis, there was one randomized and controlled trial (HORTIS) that showed positive effects of HBOT.[227]

We initiated a prospective cohort study in collaboration with the urology department at Shalgrenska University hospital and accepted patients with cystitis or proctitis for HBOT. We aimed to evaluate patient-perceived symptoms using EPIC. Hence, Paper I was a pilot study to evaluate not only the clinical effect of the treatment but also the referral and treatment protocol and the feasibility of EPIC for this purpose.

Paper I indicated the alleviation of patient-perceived symptoms after HBOT for both proctitis and cystitis. Previous studies on radiation-induced cystitis were mainly focusing on hematuria.[206,216-219,237] The knowledge regarding the effects on other symptoms and milder cases was scarce.[238] Since EPIC also covers other symptoms, such as urgency, leakage, pain, frequency, and incontinence, Paper I added valuable knowledge regarding symptoms other than bleeding. Some studies have reported long-term data (2–5 years),[225] but most studies reported one-year data.[137] We followed our cohort for two years, and the results were stable at follow up.[239] The lack of a control group was the major limitation in this study.

We concluded that EPIC was a feasible instrument for the assessment of patient-perceived symptoms. It can be used for both male and female subjects, and it covers both qualitative and quantitative symptoms.[134] EPIC only requires input from the patient and can hence be used for long-term evaluation without clinical assessment. Furthermore, EPIC is validated and has been used for over 20 years.[240] Previous studies have reported results from healthy individuals and data on minimally (clinically) important differences (MID) for each domain.[241-243]

5.1.2 WHO DOES HBOT HELP?

When the individual results were assessed, some patients improved markedly, while others did not improve at all. With the selection of patients used in Paper I

and Paper IV, the responses were divided into three groups: markedly improved or fully recovered, improvement exceeding MID, and non-responders. Roughly, our results show an equal distribution of patients in each group. This raised a question regarding the selection of patients. One factor that might affect outcomes is the time from radiation to HBOT. We are aware of one study that assessed time as a factor.[219] It assessed time from *symptoms* to HBOT, with a cut-off at six months, revealing that the effect on hematuria from HBOT was larger in the group that received early treatment.[219] Other factors that might affect the outcome of HBOT are gender, age, previous surgery in the pelvic region, radiation dose, severity of symptoms, and pathological changes. We are not aware of any study that has assessed the effects on the outcomes of these confounding factors. Interestingly, Clarke et al. noted that non-responders may experience cancer recurrence or other persistent symptoms, making it important to follow-up with non-responders.[227]

Today, there is no way to assess responses to HBOT after one or a few treatment sessions. The effects of HBOT might not be evident until after several weeks of treatment,[37] and some patients only report improvement several months after the end of HBOT. Improved selection of patients and early assessment of effects would help to target the treatment to patients who respond and to avoid treating those who do not.

In conclusion, HBOT seems to be effective for most patients with late radiation-induced cystitis or proctitis, but individual responses vary. Other causes for patients' symptoms should be excluded before HBOT is administered.

5.1.3 WHY DOES HBOT HELP?

Radiation therapy gives rise to oxidative stress with a subsequent depletion of cells and blood vessels, inflammation, fibrotic healing, and impaired organ function.[244] HBOT seems to counteract several of these processes and, in Paper II, we showed that oxidative stress is markedly reduced when HBOT is applied to the previously irradiated urinary bladders of rats. Paper III showed that cellular death can be reversed by HBOT. These findings are in line with previous reports.[81,245] Blood vessels and cells are restored via the upregulation of angiogenetic factors such as HIF–1α and VEGF and the increased recruitment of stem cells.[154] Most of the research demonstrating these changes has been conducted using animal models. Little is known about the histo- and biochemical changes in the human urinary bladder after HBOT for late radiation-induced injuries.

HBOT leads to a fast and general elevation of ROS in the body. A slightly slower, but equally general response, with elevated levels of antioxidants, is also seen during and after HBOT. ROS fall rapidly after end of HBOT, while it takes a longer time to normalize the upregulated antioxidative response system. In tissue

with late radiation-induced injuries, inflammation and relative hypoxia leads to locally elevated ROS-levels. The general but temporary abundance of antioxidants after HBOT may be able to suppress the chronic but locally elevated ROS-levels in irradiated tissue. This might explain why repeated HBOT sessions eventually break the ROS-dependent vicious circle that radiation therapy initiated.

5.1.4 IS HBOT BETTER THAN STANDARD CARE? – PROTOCOL FOR PAPER IV

The study group that published HORTIS had initiated a double-blinded study on cystitis and the hyperbaric department at Sahlgrenska University Hospital /Östra in Gothenburg joined this study. The study was terminated prematurely.[246] Low recruitment rate was one contributing factor. Many patients declined participation due to the risk of being subject to sham treatments for 6–8 weeks. Fortunately, an EUBS meeting was held just days after the termination of the HORTIS study. Representatives from all Nordic hyperbaric chambers were at this meeting, and it was decided that a joint study should be initiated: RICH-ART. We aimed to answer as many of the previous questions as possible and, at the same time, design a pragmatic study protocol. RICH-ART was randomized and included a control group. The study was stratified for gender, previous surgery in the pelvic region, and time from radiation to HBOT. Several potential confounding background factors were collected. Biopsies were taken before randomization and at the final assessment. A post-study five-year follow-up was included.

RICH-ART was not blinded, since previous attempts had proven to be unsuccessful. The control group was offered HBOT after the final assessment in the study. These are limitations, but the design with late assessment may dilute the placebo effect that the blinding aims to counteract. Indeed, well-designed, un-blinded, controlled studies may render equally valid outcomes as blinded studies and ethical issues arise when sham treatments are introduced in studies assessing the effects on conditions in which the treatment modality has already been established in clinical practice.[247-249] Since HBOT initiates processes that may continue for months after the end of treatment, we choose to evaluate the primary endpoint around six months after the end of HBOT, not directly after the end of treatment, as Clarke et al. did. We believe that a design with a delayed assessment contributes to attenuating the placebo effect. Also, the expectancy before evaluation allowed for the natural progression of the condition in the control group.

The confounding factors for which the study was stratified did not influence the effects of HBOT in Paper IV. The control group in RICH-ART received HBOT after visit 4. This means that a larger group of patients treated with HBOT were included in the post-study follow-up. We plan to re-assess several cofounding factors when the post-HBOT data are available for all patients.

Patient-perceived symptoms, assessed with EPIC, were used as the primary endpoint variable in RICH-ART. EPIC is highly sensitive, and even mild symptoms will reduce the overall score. The study has been validated and has yielded data on normal values for different age groups.[240] For some domains, EPIC scores dropped from close to 100 in lower age groups to under 90 for those aged over 60 years.[241] We used the mean total urinary EPIC score as both a primary endpoint and as an inclusion variable. In using EPIC as an inclusion variable, we sought to exclude patients with mild symptoms that might have been caused by age-dependent factors, such as reduced elasticity of the urinary bladder, rather than radiation-induced injuries.[108,240,250] We set this level at an EPIC score of 80 and considered patients with a lower score to have significant symptoms, whereas patients with a score over 80 were considered to have trivial symptoms. However, even mild symptoms can affect HRQoL, and if such symptoms are caused by late radiation-induced injuries, HBOT might be effective, even if the baseline EPIC score exceeds 80.[240,241,251]

In Paper IV, we defined MID as a change in the EPIC score exceeding 0.5 standard deviations of the total baseline EPIC score (MID = 9). This is an established method when the clinical assessment of MID is lacking.[252] However, MID has been specifically assessed for different domains of EPIC and has been found to be 6–9 for incontinence, 5–7 for irritability and obstruction, and 4–6 for bowel symptoms.[241] This suggests that we might have underestimated the effects of HBOT by setting MID too high in Paper IV. It also lends further support to the idea of treating patients with milder symptoms and a score over 80 on EPIC, since there is room for improvement for these patients. Furthermore, MID cannot be applied on an individual level, since individual baseline values and psychological factors impact each patient's perceived difference in symptoms and their impact on HRQoL.[253,254]

In RICH-ART, objective findings and underlying mechanisms were also assessed, the former via an evaluation by a urologist, and the latter via biopsies taken from the urinary bladder. We are not aware of any study on the human urothelium in this setting.

Due to the critique on the external and internal validity of some previous studies, noted both in our own HTA analysis and by reviews of the field, we established several quality assurance procedures.[137,213,255] An e-CRF was created and used for data capture during RICH-ART. The study was monitored, had a complete audit trial, and had no outstanding queries in the clean file. We believe this ensured the quality of the data and the accuracy of the results.

5.1.5 WHAT HAPPENS IN THE URINARY BLADDER? – PAPER II

Paper II was designed to assess the effects of HBOT in irradiated urinary bladders using a rat model. The rat model has successfully been used in previous experiments and has proven to be feasible for assessing radiation-induced injuries.[256] A small hyperbaric research chamber that could accommodate five rats was built for the study.

The animal study was set up to test the hypothesis that HBOT would reverse the radiation-induced responses of oxidative stress and immune activation in the urinary bladder. Physiological changes with an increased frequency of micturition and a reduced volume of urinary bladder have been assessed in this animal model using a similar radiation protocol.[257] The time from radiation to HBOT was set to 14 days, making this a study on sub-acute rather than late radiation-induced injuries. It is difficult to make direct comparisons between humans and rats, especially when it comes to long-term effects. The cellular turnover rate is higher in rats, and they do not develop fibrosis to the same extent as do humans.[258,259]

HBOT was given twice daily, to save time, for 10 days. Twice daily HBOT treatments for rats have been used before.[260] The number of HBOT sessions were sufficient to demonstrate a significant result, but future studies may want to evaluate the effects after fewer sessions to better understand the dose-dependent effects for the results seen in Paper II.

We assessed the bladders one day after the HBOT protocol was finalized, i.e., 29 days after irradiation. No major morphological differences were seen between the groups. Although rats are less prone to developing fibrosis compared to humans, the lack of morphological changes may also be explained by the relatively short timeframe between radiation and assessment.[250,261] We also assessed angiogenetic factors such as HIF1-α and VEGF. HBOT activates and stabilizes HIF1-α, which leads to increased cellular proliferation.[262] Unpublished data from our group show that HBOT may also affect HIF1-α and enhance VEGF expression and angiogenesis in irradiated urinary bladders (unpublished data).

Paper II was conducted at the same time as RICH-ART was including patients. The results and experiences from Paper II have been used to further define what analyses may be useful regarding biopsies from the urinary bladder taken in the RICH-ART study.

5.1.6 CAN HBOT BE USED PROPHYLACTICALLY?

HBOT is already used prophylactically before dental extraction in patients with osteoradionecrosis in the jaw.[2] While this is a form of prophylactic treatment, it merely aims to limit the damage of an intrusive procedure in already damaged

tissue.[263] Paper II shows that some sub-acute changes initiated by irradiation may be alleviated by early HBOT before morphological changes are seen. This raises the question: Can prophylactic HBOT weeks or months after radiation therapy reduce the incidence and severity of late radiation-induced injuries? With the reduced incidence of adverse effects, the radiation dose may be increased to achieve better cancer treatment results.

5.1.7 CAN CELLULAR DEATH BE AUGMENTED? – PAPER III

Variables such as dose of radiation, time between radiation and exposure to HBOT, the duration of HBOT, the pressure used, and the number of HBOT sessions can all be altered and can tentatively produce different effects and outcomes. With hundreds of different protocols, we were unable to proceed with an animal model. Hence, we examined the feasibility of a cellular model, since cellular models allow for studies of specific factors in a well-defined and controlled environment. Cellular models can also be adjusted in order to study specific pathways.

The focus was on establishing a model for future cellular experiments in which specific effects and pathways involved in irradiation and HBOT can be assessed. We used human urothelial and endothelial cells and were able to establish a protocol that reversed cellular death in both cell lines. These protocols can be used for future studies to assess the dose-dependent effects of radiation and HBOT, as well as for studies on specific cellular pathways and mediators.

5.1.8 RICH-ART IS NOT FINALIZED – BEYOND PAPER IV

The first part of RICH-ART has been finalized, but apart from a five-year follow-up, additional data need to be assessed. The control group was offered HBOT directly after visit 4. Further analyses of confounding factors will be conducted when all patients in the study have completed HBOT. An analysis of biopsies concerning oxidative stress, fibrosis, angiogenesis, immunological activity, and other factors will also be performed. The five-year follow-up includes yearly assessment with EPIC and SF–36, and long-term effects will be assessed when the data are available (estimated to be at the end of 2024).

It would have been desirable to have a longer follow-up for the control group before offering HBOT. However, HBOT was a part of clinical practice at most centers, and it was considered impossible to withhold the control group HBOT for too long. Six months was believed to be sufficient for the effects of HBOT to stabilize and allow for natural progression in the control group. The risk of patients declining participation increases if the waiting time is too long, with an extension to 12 months believed to create problems with inclusion.

5.1.9 WHAT IS THE OPTIMAL DOSE OF HBOT?

As outlined in the introduction (1.5.10), there is little dispute over the optimal treatment pressure: 200–250 kPa. This pressure can only be marginally increased due to the risk of neurological oxygen toxicity at higher pressures. It is unlikely that the desirable effects will be increased at lower pressures, and hence there is no need to decrease the treatment pressure.

Most patients in RICH-ART received 40 sessions of HBOT, but at one center only 30 sessions were administered. In a preliminary analysis, no differences in the primary endpoint were observed between patients treated with 30 and 40 sessions. Previous studies have mostly used 30–40 sessions, although some studies have used even more.[137] The question about dose was coupled with the question about responders. Further studies on dose-dependent effects are needed. The value of iteration of the treatment months or years after the first session has also been poorly investigated.

5.1.10 WHAT ABOUT SEXUAL FUNCTION?

Sexual and hormonal function was not assessed. Symptoms from these domains are common, and very few studies have assessed the effects of HBOT.[264,265] We are aware, however, of one study currently being performed with this focus: "Hyperbaric Oxygenation Treatment and Quality of Life," Haukeland University Hospital, Bergen, Norway.[266] This study is focusing on female patients for whom late radiation-induced injuries frequently lead to sexual dysfunction.[267,268] This is also a common problem for male patients with a history of pelvic radiation therapy.[240,241] More studies on sexual dysfunction after radiation therapy and the use of HBOT are needed.

5.1.11 IS IT WORTH IT?

Cost effectiveness is important, since resources are limited. The cost of one HBOT elective session is around €200 in Sweden. Visits to the emergency room and costs for admission and intervention can easily exceed the cost for a complete HBOT protocol. This was illustrated in a study in which the total health care costs for a patient with radiation-induced cystitis were compared before and after HBOT.[269] There was a reduction of the yearly health care cost one year after HBOT, even when the cost of HBOT was included in the post-treatment total. It therefore seems like HBOT can save money by reducing the need for emergency and in-hospital care. We have very limited knowledge regarding other costs and potential savings, i.e., reduced sick leave. There is also a need for studies that investigate whether patients perceive the treatment as "worth it" or not, regardless of the economic aspect.[256,257]

5.1.12 LIMITATIONS

Although enough to generate significant results, the number of included patients were small in both clinical papers, 39 patients in Paper I and 79 in Paper IV. This limits the level of sub-group analyses that can be made and make analyses of confounding factors hard.

Although included in the study design of RICH-ART, the five-year follow-up has not been finalized. Hence it is too early to say anything about long-term effects based on the papers in this thesis.

Adverse events were recorded during HBOT in RICH-ART but was not included in the long-term follow-up. We are not aware of any report on increased risk for recurrence of cancer or new tumors after HBOT, on the contrary, several reports have not found any such link.[212,270] However, this aspect needs to be monitored in future studies and registries.

5.1.13 STUDY POPULATION AND ETHICAL CONSIDERATIONS

The study populations in the clinical papers (Paper I and IV) may have been affected by doctor's and patient's delay or for referral. Also, patients with symptoms of late radiation-induced injuries were handled by doctors from different specialties. Knowledge of HBOT varied greatly between individual doctors, patients, and the general population.[136] This might have contributed to the selection of patients with more severe symptoms, mainly referred to HBOT from urology departments at university hospitals.

Patients with catheter or other urinary deviations were excluded from RICH–ART. This was because the EPIC form contains questions that these patients are unable to answer, and hence no valid EPIC score can be calculated.[240] However, these patients suffer from the same condition as those included in RICH-ART, and it is thus reasonable to believe that HBOT will improve patient-perceived symptoms in this group of patients as well. Hence, urinary deviation or catheters should not be viewed as contraindications for HBOT in late radiation-induced injuries. Patients with severe hematuria were also excluded. HBOT without delay was considered the standard of care in these severe cases by the urologists.[137,209,219] Hence, it was considered unethical to randomize these patients, which would have involved treating one group conservatively.

We used an animal model in order to assess the effects of HBOT in the irradiated urinary bladders of rats. Using smaller animals, such as mice or hamsters, would have made it difficult to aim the radiation correctly and would not have provided sufficient tissue material for the planned assessments. Using larger animals would have required more resources and a larger research hyperbaric chamber.

Furthermore, the rat model has already been used and proven to generate both microscopical changes and functional impairment.[256,257]

5.1.14 A NORDIC HBOT REGISTRY

Several questions have been raised in the discussion that illustrate future perspectives in this field. Some questions may be answered when more data from RICH-ART become available, but many others will require new studies. However, additional studies can be costly and could require numerous resources. Well-designed registries can give answers to many of the outstanding questions in this field. Prospectively collected data from several centers can be used to evaluate confounding factors, effects on sexual function, dose-dependent effects, long-term outcomes, etc. The Nordic hyperbaric research group, created for the RICH-ART study, already designed an HBOT registry in 2016. It has not yet started data collection due to a lack of funding, but it is planning to start the enrollment of patients at the end of 2020.

5.1.15 FINAL REMARKS

HBOT can alleviate patient-perceived symptoms of late radiation-induced cystitis and proctitis and improve HRQoL. HBOT can also reduce macroscopic changes of the urinary bladder as well as reverse the oxidative stress and some of the downstream effects caused by radiation therapy. The results add further support for the efficacy of HBOT for late radiation-induced injuries, which may not only help afflicted patients but might also increase the effectiveness of future cancer treatments.

6 CONCLUSION

Paper I

HBOT may be an effective and safe treatment for late radiation-induced soft tissue injuries in the pelvic region.

Paper II

In an animal model for radiation cystitis, significant elevation of oxidative stress, antioxidants, and pro-fibrotic factors were observed, and these were reversed by HBOT.

Paper III

Cellular death induced by irradiation can be reversed with one session of HBOT for UROtsa cells and, in a dose-dependent manner, for HUVEC cells.

Paper IV

HBOT is an effective and safe treatment for late radiation-induced cystitis and can improve HRQoL.

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APPENDIX