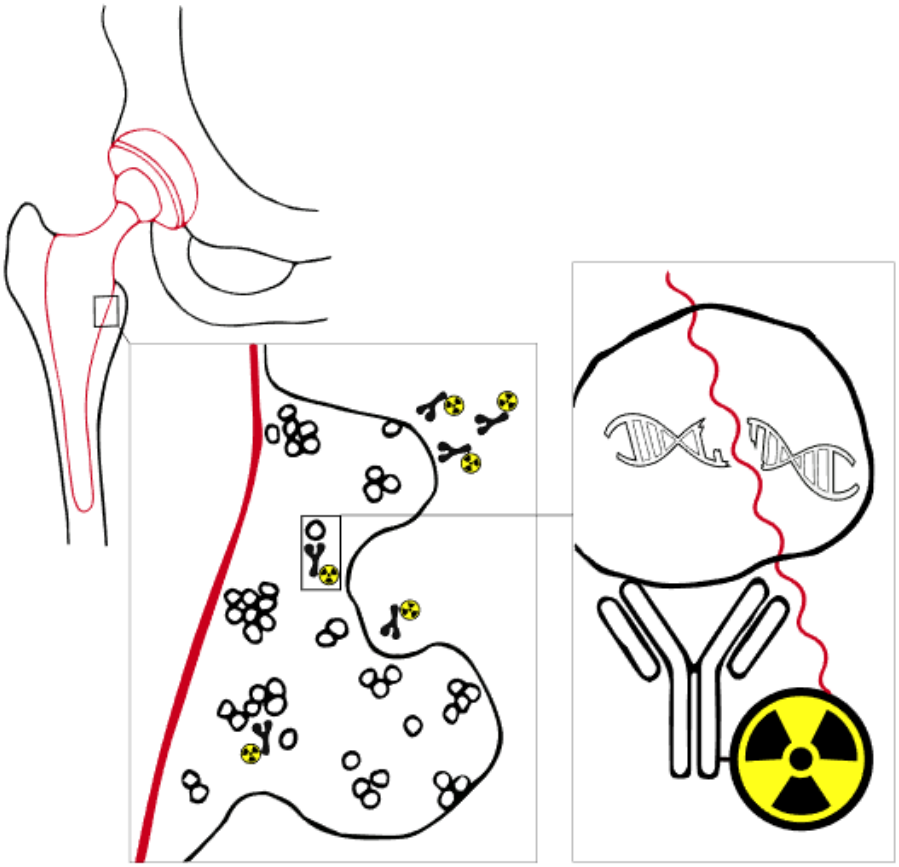
Treating Infections with Ionizing Radiation: A Historical Perspective and Emerging Techniques



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Abstract

Background: Widespread use and misuse of antibiotics have led to a dramatic increase in the emergence of antibiotic resistant bacteria, while the discovery and development of new antibiotics is declining. On top of that, implant associated infections such as periprosthetic joint infections are even more difficult to treat due to biofilm formation. Alternative treatment options are needed to treat these infections in the future. This article aims to provide a historical overview and future perspective of radiation therapy in infectious diseases with a focus on orthopedic infections.

Methods: A systematic search strategy was designed for three academic databases, Pubmed, Embase and Cochrane, to select studies that used radiation as treatment for bacterial or fungal infections. A total of 170 potentially relevant full-text publications were independently reviewed, of which 129 focused on external radiation and 41 on internal radiation. Due to the large number of studies, several topics were chosen. The main advantages, disadvantages, limitations, and implications of radiation treatment for infections were discussed.

Results: In the pre-antibiotic era, high mortality rates were seen in different infections such as pneumonia, gas gangrene and otitis media. In some cases, external radiation therapy decreased the mortality significantly but long-term follow-up of the patients was often not performed so the long term radiation effects, as well as a potential increased risk of malignancies could not be investigated. Internal radiation using alpha and beta emitting radionuclides show great promise in treating fungal and bacterial infections when combined with selective targeting through antibodies minimizing possible collateral damage to healthy tissue.

Conclusion: The novel prospects of radiation treatment against planktonic and biofilm-related microbial infections seem feasible and could improve treatment outcomes. However, the possible risks involving radiation treatment must be considered in each individual patient.

Introduction

For more than a century, radiation has been used as a treatment modality for a wide range of diseases. Its usefulness in diagnosis and oncological treatment is currently undisputed, but in the early 20th century radiation was also commonly employed to treat infections. Infections were deadly at that time and without other effective treatments available and limited knowledge of possible side effects, radiation therapy

was used extensively. In the 1940s, radiation treatment became obsolete with the discovery and availability of antibiotics. Today, the war against infections is still ongoing. Widespread use and misuse of antibiotics have led to the emergence of antibiotic-resistant bacteria, while the discovery and development of new antibiotics is rapidly declining.[1]

The field of orthopedic surgery is in dire need of such novel treatments. Total joint replacements are a common, last-resort treatment for degenerative joint disease, but unfortunately, 1-4% of the patients develop a periprosthetic joint infection (PJI).[2] PJI is difficult to treat as bacteria form a biofilm on the prosthetic material. This hinders the host immune system, but more importantly, the bacteria in a biofilm are mostly in a metabolic inactive or dormant state and therefore not susceptible to most antibiotics.[3]

In the Medical Center Utrecht, patients with PJI are generally treated with Debridement, Antibiotics and Implant Retentions (DAIR), 1-stage revision or a 2-stage revision. These last two will be shortly discussed. The one-stage revision consists of removing the infected material, debridement and irrigation of the infection site followed by implantation of a new hip or knee prosthesis under the same anesthetic. The two-stage revision consists of two surgical interventions. In the first operation, the infected prosthetic joint is removed along with all the material suspected to be infected. After this, the surgical site is cleaned by debridement and irrigation and an antibiotic loaded spacer is implanted. This spacer stays in situ for 6 to 8 weeks along with systemic antibiotics. In the second and final stage of the revision, the surgical site is irrigated and the new hip or knee prosthesis is implanted. In both surgical revisions multiple intraoperative samples were taken for microbiological diagnostics to identify the causative pathogen. However, not all infections are eradicated in patients treated for chronic PJI after one- and two-stage revision surgery.

Data of 247 consecutive patients that were treated in the UMC Utrecht for PJI between January 2011 and January 2017 was collected. A total of 144 patients that were treated with a one- or two-stage revision after hip (n=94) and knee (n=52) arthroplasty were eligible for analysis. Baseline characteristics (Table 1) included patient's characteristics such as age, sex, BMI and ASA-score and Prosthesis and infection characteristics such as implant location and type of revision. Microbiology characteristics include pathogen species, number of pathogens and antibiotic resistance. Per patient, multiple samples were taken peroperatively and used for routine bacterial culture. Samples were cultured up to 14 days to identify slow growing bacteria. At least 2 positive cultures were needed to correctly identify the causative organism. A polymicrobial infection is when two or more pathogens were identified.

Table 1 Baseline Characteristics	Total	Success	Failure
	n = 144	n = 112	n = 32
Patient characteristics			
Age	70,8 (44-92)	70,0 (44-92)	73,4 (54-84)
Sex (M/F)	83/61	64/48	19/13
BMI (N=145)	27,5 (18-43)	27,6 (18-38)	27,1 (19-43)
ASA-1	17	13	4
ASA-2	90	73	17
ASA-3	35	25	10
ASA-4	2	1	1
Previous orthopedic surgery			
Hip	58	44	14
Knee	29	23	6
Prosthesis and infection characteristics			
Total hip revisions	92	71	21
Total knee revisions	52	41	11
Revision type			
1-stage revision	28	20	8
2-stage revision	116	92	24

Patients remained in clinical follow-up for at least one year. In the last outpatient clinic visit, assessments of infection signs were done by the treating physician. Eradication failure was determined as unplanned subsequent surgery because of persistent infection, use of suppressive antibiotics or signs of infection at one year follow-up. It was analyzed whether the outcome was influenced by treatment type or causative

pathogen.

In the entire population of 144 patient there were 28 one-stage revisions (21 hips vs 7 knees) and 116 two-stage revisions (71 hips vs 45 knees). The mean age was 71 years with a mean BMI of 27. Patients could have had one or multiple DAIR, one- or two-stage procedures and multiple antibiotic treatments prior the surgery in the UMCU.

108 patients had a positive identification of a causative pathogen from the samples taken perioperatively. A total of 65 pathogens were identified of which *Streptococcus epidermis* was the most frequently found in both hip (N=16) and knee (N=9) infections followed by *Staphylococcus aureus* for hip (n=6) and knee (n=7). (Table 2.2) 75 *of 108 culture positive patients were monomicrobial versus 33 polymicrobial infections*. Polymicrobial infections could host up to five different bacterial species. One-stage revision with a polymicrobial infection had an eradication rate of 80%, compared to the two-stage revision the eradication rate was 86%. In general the *S. Aureus* had the lowest infection eradication rate of 69% in the two-stage group. The one-stage did not have any pathogen outliers associated with a low eradication rate. *S. Aureus* was also the pathogen in which the biggest differences were found in revisions when comparing knee to hip. In the knee-group only a success percentage of 57% was gained, while 83% was gained in the hip group.

Table 2.2	**totaal**	**hip**	**Knee**
None	36	18	18
Polymicrobial	33	24	9
Monomicrobial	75	50	25
Staphylococcus epidermidis	25	16	9
Staphylococcus Aureus	13	6	7
Other	37	28	9

There was no difference in outcome when the pathogen had developed antibacterial resistance. The eradication rate for infection with any form of bacterial resistance was 75% and for non-resistant infections 79%. There was a slight difference between the

one-stage and two-stage group. Resistant bacteria were better eradicated in the two-stage group with 77% compared to the one-stage group 69%. Mostly the *Staphylococcus epidermidis* and *S. Aureus* were the bacteria that build the most resistance against antibiotics.

As stated above, patients with PJI get prolonged antibiotic treatment combined with multiple surgeries with- or without implant exchange to fight the infection. Despite this intensive treatment, outcome is often still unpredictable. On top of that, this mostly elderly population often has multiple comorbidities and multimodality treatment is needed. In this regard, PJI patients are not dissimilar to oncology patients, with comparably high morbidity- and mortality rates. The 5 year mortality of PJI is even higher than that of most forms of prostate-, breast- and thyroid cancer.[4,5] Ionizing radiation may play a role in infectious diseases, as it does in oncology.

Ionizing radiation therapies of the past, like x-ray- or radioactive iodine therapy, damaged a large area around the region of interest. However, recent advances in both external and internal radiation techniques make these therapies potentially more accurate. In external radiation treatment, these advances include intensity-modulated radiotherapy, as well as novel technologies like MR Linac.[6] Similarly, in internal radiation treatment radioimmunotherapy (RIT) has allowed the delivery of cytotoxic radiation to specific target cells, through the coupling of antibodies and radioisotopes.[7] The same concept could be applied to treatment of infection, by coupling the radioisotopes to an antibody that targets bacterial cells or biofilm antigens.[8] With these advances in therapeutic nuclear medicine strategies, a re-evaluation of their merits in infection treatments seems warranted. This article therefore aims to provide a historical overview and future perspective of radiation therapy in infectious diseases with a focus on orthopedic infections.

Methods

A systematic search strategy was designed for three academic databases, Pubmed, Embase and Cochrane, to select studies that used radiation for treatment of bacterial or fungal infections (Appendix 1). Studies were independently screened in two stages: screening of titles and abstracts, followed by the retrieval and screening of full-text publications. Inclusion criteria as described in Table 3 were used. No restrictions were set on publication date, due to the nature of this historic review and to ensure that no historic knowledge was overlooked. Reference screening and citation tracking of the included publications was performed to find additional publications. The included full-text publications were then divided into two main groups: studies investigating external radiation therapy and publications investigating internal radiation therapy. Since the included publications differed strongly in scope, disease and patient populations, results were clustered by their organ system or disease group.

Results

Of 16,302 studies, 170 potentially relevant full-text publications were independently reviewed. In the external radiation group, 129 publications were included, for the internal radiation group, this was 41. Due to the large number of studies, the following topics were chosen and described in detail below: external radiation treatment for pneumonia, gas gangrene and otolaryngological application and internal radiation treatment for bone tuberculosis and ankylosing spondylitis, helicobacter pylori and radioimmunotherapy for bacteria and fungus. Unfortunately, there were no suitable articles for radiation therapy on osteomyelitis that could be included. However, the results of the other infection groups can indirectly correlate to orthopaedic infections.

External Radiation

Discovery of X-rays

In 1895, Wilhelm Röntgen was the first to describe the existence of X-rays.[9] Following the publication of a radiograph of his wife's left hand (Figure 1), this new technique was

welcomed with great enthusiasm. A few years later, the first therapeutic uses were described for infectious diseases.

Pneumonia treated with X-ray

Before the advent of antibiotics, pneumonia was a disease known for its high mortality.[10] Musser and Edsall, performing clinical experiments with x-rays, found that this radiation markedly improved condition and disease progress of leukemia patients, which they hypothesized was due to an increase in metabolic processes in tissues.[10] Unresolved pneumonia was, in their opinion, also a situation in which the body could not adequately metabolize the unresolved exudate that was left in the lungs. Based on this theory, they treated a patient who suffered from a 1 month old unresolved pneumonia with x-ray treatment for 5 minutes daily during 5 days. At the end of the week, the pneumonia had completely resolved.[10] Following this publication, multiple publications were published that also investigated the merits of x-rays in unresolved pneumonia, with good clinical results.[11,12] Krost et al. then investigated x-ray treatment for pneumonia in 12 children with unresolved pneumonia.[13] These patients had symptoms for as long as 3-6 weeks before the first x-ray treatment was given. After 1-2 x-ray treatments, (5 mA, 5min, spark gap 7.5 inches, distance 8 inches, 3 mm Al and 4mm leather filter) 11 cases of pneumonia (92%) resolved within several days, the clinical situation often improved after hours. Powell et al. continued research of x-rays in the 1930's, his cohort of adults showed a decreased mortality of 6.7% (9/134 patients), a sharp improvement from earlier mortality rates for pneumonia.[14] In that study, patients were alternatively included in the x-ray group or the control group, but after seeing the marked reduction in mortality in the x-ray treatment group, all control patients were subsequently treated with x-rays (all patients received 250-350 röntgen of 0.3 angstrom unit). A few years following Powell's research, sulfonamides, the first antibiotics, were used as standard treatment for pneumonia, and use of x-rays fell out of favor. Research, however, was continued for patients who did not respond to, or did not tolerate sulfonamide therapy. In one such study, 22 out of 29 patients (75.9%) who showed no response to sulfonamides, recovered completely with x-ray therapy (120Kv,

40cm distance, 3mm Al filter, 200 r single-dose for a maximum of 3 doses).[15] Some short-term adverse effects were shown by several authors, namely convulsions and cyanosis when the single session radiation dose exceeded 10 Gy.[16,17] These complications often resolved, and therapy was still effective in these patients. Unfortunately, none of the authors performed long-term follow-up of their patients, so the long term radiation effects, as well as a potential increased risk of malignancies could not be investigated. For a comprehensive review of the clinical and animal literature on x-ray use in pneumonia, we direct the reader to the comprehensive review by Calabrese and Dhawan.[18]

Gas Gangrene

Gas gangrene, or clostridium myonecrosis, is a destructive soft-tissue infection caused by anaerobic clostridium bacteria. The micro-organisms that are often associated with severe trauma or contaminated wounds thrive in low-oxygen environments and rapidly destroy muscle tissue while producing gas in the tissues. Severe pain, edema and/or bullae, an unusually rapid tachycardia, and palpable soft tissue crepitations are all clinical signs that point to the presence of gas gangrene.[19] Before the antibiotic era, surgery, namely amputation, was the only treatment, and mortality was around 50%.[20] Radiologist Kelly reported in 1931 his experience with treating gas gangrene with x-rays and found a mortality of only 2 in 8 patients, without the need for further amputation after x-ray treatment (6-7 doses of 3min; 5-inch spark gap, 5mA, 15 inch distance, 0.5mm Al filter). He described this in his paper in one patient: "*The laboratory cultures were positive for Bacillus welchii, and x-rays films showed considerable gas in the soft tissues. Amputation was advised by consultants, but action was deferred to see the effects of the other treatment. Serum* [equine serum containing antibodies against one or more clostridium species] *and x-ray therapy were administered. No amputation was necessary and the patient was dismissed after seven weeks' hospitalization*".[21] Following Kelly's initial success, many studies were performed over the years, with the majority showing excellent results. In a review and meta-analysis of the case series literature, Kelly and

Dowell showed that a combination of surgery, serum therapy and x-ray treatment (different radiation regimes were used during this study) resulted in a 11.5% mortality (42/364 patients) compared to a 35-50% mortality rate when only surgery and serum were evaluated together.[20] In a subgroup of x-ray patients who received multiple x-ray treatments, mortality was even lower, at 5.9% (17/288 in patients with ≥ 3 x-ray treatments). In a subgroup that underwent only x-ray treatment without serum therapy, mortality was 4.7% (2/42 patients) and no amputations were necessary. How x-rays halted the gas gangrene infection was never elucidated, although it was generally known that the relatively low radiation dose was not able to destroy the bacteria directly. More likely hypotheses that were proposed included the possibility that radiation causes local vessels to dilate, increasing oxygen supply to the infected tissue and thus diminishing the potency of anaerobic bacteria, as well as the possibility that radiation stimulated either the proliferation of immune cells or the release of bactericidal products from lymphocytes.[22,23] It must be noted that some authors did not find x-rays to be effective,[24] and that the promising mortality figures could have been the result of selection bias as well as an improved standard of care for these infections over time.[25]

Otolaryngological applications

Before the advent of tympanostomy tubes, otitis media was a major health problem in school children. Following upper respiratory tract infections, tissue in the nasopharynx swells and blocks the Eustachian tube, thus blocking the outflow of middle ear secretions, which may become infected and cause conductive hearing loss. Blockage of the Eustachian tube may also be caused by swelling of the adenoid tissue of the nasopharynx.[26] Treatment in the past consisted of paracentesis, adenoidectomy or surgical removal of tissue surrounding the Eustachian tubes, although these therapies were often ineffective. The resulting chronic hearing loss had a deleterious effect on the development of normal hearing and speech of children.

Early in the 20th century, x-rays were proposed as a viable treatment to otitis media caused by Eustachian tubes blocked by lymphoid tissue, as it was already known that these tissues were very radiosensitive.[27] Beattie et al. found in 1920 that patients suffering from chronic otitis media with symptoms of mastoiditis showed clinical improvement after diagnostic mastoid x-rays. Out of 14 chronic patients, 9 improved after only 1-3 sessions with 180 seconds of x-ray exposure.[28] Similar results were found by other studies over the years.[29]

Crowe and Baylor, happy with the effect that radiation had in reducing lymphoid tissue around the Eustachian tube, proposed that radiation could be applied much more locally compared to x-ray through nasal application of a small radioactive radium or radon source, which would cause much less systemic radiation.[30] Through covering the applicator with brass, all alpha- and most beta-radiation was filtered. Gamma rays were emitted that mimicked the x-ray treatment, but applied only locally, where it was needed. The technique was optimized by Crowe and colleagues, and a nickel-copper alloy was used instead of brass to cover the applicator, so that more beta-radiation was emitted that decreased the necessary application time and reduced the gamma-radiation load on tissues other than the nasopharyngeal lymphoid tissue. The treatment differed between studies but often consisted of 1-4 sessions of application with around 25-50 mg ^{226}Ra sulphate for 8-15 minutes (~5 Sv at lymphoid tissue over 6 sessions, total dose in surrounding tissues estimated to be 36-142 Sv).[31,32,33] The efficacy of the treatment was excellent, symptoms decreased within days, and the radium treatment was used in many children, but also in thousands of air force pilots and submarine personnel who had undergone baro-trauma.[34]

The positive results in children were illustrated in a randomized controlled trial by Hardy and Bordley, which consisted of over 1000 school children with conductive hearing loss who were randomized in groups that received three sessions with an applicator containing either radium or a placebo, blinded to patient and physician.[35] In

the subgroup with greatest hearing loss (i.e. the group with large lymphoid tissue overgrowth), hearing improved significantly greater with radium therapy compared to control treatment, and lymphoid tissue was significantly reduced. Interestingly, mild hearing loss in the control group improved markedly over the years as well, from which it was concluded that radium therapy should only be performed in cases in which hearing loss is found as a result of Eustachian tube dysfunction, because in most other cases, the condition also improved without treatment.

Over time, physicians became more concerned on the potential long-term health effects. An increase in cancer risk was suggested by some studies that followed children who had received radiation for benign conditions during childhood.[36,37] But these increased cancer risks were never unequivocally shown in cohort studies that investigated patients treated with nasopharyngeal radium. A cohort by Ronckers et al. found no increase in head and neck- or thyroid malignancies in a large cohort of over 4000 patients, although the incidence of breast cancer and non-Hodgkin lymphoma was slightly elevated.[33] Another study by Yeh et al. found no significant increase in the incidence of malignancies in a cohort of more than 1700 patients with around 40 years of follow-up.[38] Loeb et al. performed a literature review of studies on nasal radium therapy that included almost 30,000 patients (of whom a large proportion was treated by Crowe and colleagues). They found no cases of malignancies that could be clearly attributed to radium treatment.[39]

Although an increased incidence of malignancies was never proven, the use of radium was not without risks. Notable was an incident in 1958 at the otolaryngology department of our own university, the University Medical Center Utrecht, where the tip from a radium capsule broke away from the applicator, and was accidentally swallowed, with the treating physician being unaware. The 5-year old patient returned home, where she threw up the capsule, which was then accidentally deposited into the chimney by her father. The charred (and radioactive) ashes were distributed outside,

thus contaminating the entire house and garden with radioactive material. This prompted a citywide emergency, the patient and her family were quarantined, and all persons who had contacted the family during the incident had to be examined both medically, and with Geiger Counters (Video 1). During the first month after the incident, parts of the house were broken down and renovated by army personnel in protective gear. The radioactive waste was dumped in the ocean, some 30 miles from the Dutch coast. A few months after the incident, a new "Radioactive substance decree" was written into Dutch law, detailing "(…) *that sources of Radium could only exceed 1 mCu if, and only if, adequately encapsulated by a shell that cannot be removed without damage* (…), *which is hermetically sealed and which is created from an indestructible material* (…)".[REF] Unfortunately, this measure came too late. The incident caused much media publicity, and with increasing fear of radioactive substances, fueled more so by the Cold War, radium therapy was quickly abandoned in The Netherlands, also partly because of the advent of non-radioactive alternatives. An in-depth description of this incident was written by Graamans.[40] The patient was said to have lived a healthy life, with no radiation-related complications.

Internal Radiation

In this review, internal radiation is defined as a systemic treatment, involving radioisotopes that deliver a cytotoxic level of radiation to a diseased site. The hypothesis of "magic bullets" that could selectively kill pathogens or cells without harming healthy tissue was first described around 1900 by Paul Ehrlich.[41] The concept of targeted radiation therapy was used from the 1900s for different infectious diseases and is described in detail below.

Thorium X

Starting from around 1912, Thorium X was used in dermatology and as a treatment for rheumatic diseases. Thorium X (Radium-224; ^{224}Ra) is a short-lived alpha-emitter (half-life of 3.6 days) and was applied topically, intravenously and orally. Around 1940,

Peteosthor was developed to successfully treat bone tuberculosis and ankylosing spondylitis.[42] The drug contained ^{224}Ra-chloride (Thorium X), platinum and red dye eosin. The hypothesis was that this short-lived bone-seeking alpha-emitter could selectively target, accumulate, and destroy the inflamed tissue. Between the 1940's, and mid-1950's, primarily children and juveniles were treated with high doses of ^{224}Ra, receiving repeated injections up to 2 MBq twice a week, often for prolonged periods of time, sometimes totaling up to 140 MBq.[43] Around 1950, Spiess and Mays questioned the efficacy of Peteosthor and conducted several in vitro and in vivo experiments. They showed that killing of *Mycobacterium tuberculosis* was seen in vitro with high doses of ^{224}Ra, but no killing was seen in vivo. Objections to the treatment were raised in the early 1950's, the primary one being that ^{224}Ra deposited in the growing skeleton of children and juveniles would cause severe damage.[43] Because of the questionable efficacy of the treatment and the introduction of antibiotics like Streptomycin, discovered by Waksman (1943), Peteosthor was abandoned as a treatment for bone tuberculosis in 1956. However, ^{224}Ra treatment for ankylosing spondylitis was changed to a low-dose scheme, with good results. It was continued until the late 90's, when non-radioactive alternatives like TNF- alpha came to the market that had less side effects.[44] After 1956, Spiess and Mays followed a cohort of 899 patients treated with high doses of Peteosthor for many years. A significant increase was seen in the incidence of bone tumors (56 cases among 899 patients, 6.2%).[42]

Iodine-131 – helicobacter pylori

Helicobacter pylori (Hp) infection is probably the most common chronic bacterial infection, present in almost half of the world population.[45] Multiple studies investigated the effect of radioactive iodine-131 (^{131}I) on Hp. ^{131}I is a short-lived beta-emitter (half-life 8.4 days) and is an important treatment modality in the management of thyroid cancer and hyperthyroidism. ^{131}I does not only accumulate in the thyroid, but also in the stomach, and could therefore potentially eradicate Hp infection.[46] In 71 patients treated for differentiated thyroid carcinoma, a pre-treatment urease breath test was

done to diagnose an Hp infection. Twenty-three patients had a negative post-treatment result and thus a significant reduction in Hp.[46] In another study, 18 of 85 patients infected with Hp who were treated for hyperthyroidism with ^{131}I showed a negative UBT after treatment, which also means a significant reduction in Hp.[47] However, no significant reduction was seen in two other studies, the first with 18 patients treated for differentiated thyroid carcinoma and the second study with 76 patients treated for differentiated thyroid cancer and 11 for primary hyperthyroidism.[48,49] These studies show that ^{131}I therapy may have an antimicrobial effect on Hp.

Radioimmunotherapy

Currently, RIT is used to treat different types of cancer, but until the 1940's, cancer treatment was mostly based around the surgical approach. That changed with the advent of molecular medicine, and with the discovery of "chemotherapy" by Louis Goodman and Alfred Gilman.[50] In the next few decades, multiple chemotherapy agents were discovered that successfully induced remission of multiple types of cancer. However, during the development of these systemic cancer drugs, significant problems, such as acute and long-term toxicities were repeatedly encountered. Therefore, a change of strategy was needed and was found in targeted-therapy.[50] The aim of targeted therapy is to specifically target tumor cells with specific antibodies or small molecules that interfere with molecular pathways related to carcinogenesis and tumor growth. In the late 1980's, researchers shifted their focus to unraveling and understanding these molecular pathways and due to innovations in technology more and more antibodies and inhibitors of specific targets were discovered.[51] While antibodies can directly affect tumor cells, they can also be used as transport vehicles to deliver agents that can destroy tumor cells (e.g. radioisotopes).[52] When antibodies are labeled to radioisotopes, a high dose of ionizing radiation can be delivered directly to the targeted cells. In the past decade, success was seen in treating non-Hodgkin

lymphoma with the only two radioimmunoconjugates approved by the FDA, ^{131}I-tositumomab and ^{90}Y-ibritumomab tiuxetan.[52,53]

Radioimmunotherapy on fungal infections

In vitro experiments showed that both planktonic cells and biofilms of *Cryptococcus neoformans* (CN) are susceptible to RIT. In vitro, CN-specific monoclonal antibodies conjugated to bismuth-213 (^{213}Bi; short-lived alpha-emitter, half-life 45 min.) caused a 50% reduction in metabolic activity of the fungal biofilm and a 70% reduction in metabolic activity of planktonic cells at a dose of 1.11 MBq (30 µCi) when compared to the control non-specific antibody conjugation.[54] In the same study, 14.8 MBq (400 µCi) rhenium-188 (^{188}Re; short-lived beta-emitter, half-life 17 h.) conjugated to CN-specific antibodies showed a reduction in metabolic activity of planktonic cells of 83%, but no reduction was seen in the metabolic activity of the biofilm.[54]

In an in vivo experiment of Dadachova et al., nine groups of 10 mice were infected with 10^5 CN cells. Multiple treatment groups were treated with intravenously administered specific antibodies bound to ^{213}Bi and ^{188}Re. A dose of 3.7 MBq (100 µCi) RIT showed a survival of 60% with ^{213}Bi and 40% with ^{188}Re on day 75 post-therapy when compared to 0% survival in the 'cold' antibody conjugates and a saline-treated group.[55] In another study with the same in vivo CN model, RIT with ^{213}Bi was compared to the antimycotic drug amphotericin. RIT was more effective in reducing fungal burden in lungs and brains, measured by CFU count in post mortem organs, where ^{213}Bi conjugates could completely clear the infection, while amphotericin could not reduce the number of fungal cells.[56]

Radioimmunotherapy on bacterial infections

The same group also used RIT to combat bacterial infections. In vitro tests with ^{213}Bi radiolabeled antibodies against *Streptococcus pneumoniae* showed minimal but significant killing when doses of 0.11-0.15 MBq (3-4 µCi) were used.[57] A higher dose

could potentially have a higher bactericidal effect. Two in vivo experiments were done with C57BL/6 mice infected intra-peritoneal with 1000 CFU *S. pneumonia*. In the first experiment, mice were treated with either ^{213}Bi specific antibodies or "cold" antibodies, one group was left untreated. After 14 days, 87% of the mice treated with ^{213}Bi survived versus 40% in the other two groups. In the second in vivo study, the mice were treated with 2.96 MBq (80 μCi) ^{213}Bi labeled specific and non-specific antibodies. Unlabeled antibodies and an untreated group were used as controls. Mice treated with ^{213}Bi labeled specific antibodies showed a 100% survival after 14 days versus 20% in the Bi^{213} bound non-specific antibody group and 60% in the unlabeled antibody and untreated group.[57]

In another study, RIT with ^{213}Bi showed prolonged survival in mice infected with *B. Anthracis* bacterial cells compared to control groups with unlabeled antibodies and PBS.[58] These results showed the therapeutic potential of RIT on infectious diseases.[59] Until now, there is no literature on using RIT to treat infections in humans.

Discussion

Throughout history humanity has battled infections and the war is still going on today. With an increasing incidence of antimicrobial-resistant bacteria, finding effective treatments has become increasingly important. In the last century, different treatments have been developed and later abandoned. However, with the new techniques possible today, and the need to move away from our dependency to antibiotics, it is not unwise to give older strategies another consideration. Also, gathered knowledge on therapies from other fields in healthcare could potentially be used to treat infections. This review aimed to provide a summary of both historical and recent advances in radiation treatment, while giving insights in how to proceed forward and learning from mistakes made in the past. Both external and internal radiation has the potential to kill bacteria and clear infections as shown in this review. However, collateral damage to healthy tissue is a major concern, especially in external radiation treatment. To treat infections

with (external) gamma-radiation, a high dose is needed to kill the bacteria. As a consequence, the risk of cancer increases in patients who are exposed to these high doses of radiation. Of course, X-ray therapy for infections largely preceded the onset of advances in linear particle accelerators and radiotherapy; therefore, radiotherapy has mostly been ignored as a potential candidate in infection treatment, especially since antibiotics were highly effective and widely available. As we are entering an era in which antibiotics are increasingly failing, development of stereotactic radiation therapy, intensity-modulated radiation therapy and MR guided radiotherapy may, in theory, prove useful as a last resort treatment for resistant infections.

In contrary to these therapeutic techniques base on gamma radiation, alpha- and beta emitting radioisotopes can also be used for infection treatment. These radioisotopes have less penetrating power but are much more destructive, especially alpha-radiation. As early as 1950, the bactericidal effect of alpha-emitting radioisotope ^{224}Ra is shown in vitro. This makes them particularly interesting to use as Paul Ehrlich's "Magic bullets" that can target bacteria or the biofilm, while minimizing collateral damage to healthy tissue. Key in internal radiation treatment for infections is to bring the radioisotopes in close vicinity to the target. For example, ^{224}Ra has bone-seeking properties as it is a calcimimetic and is therefore incorporated into bone with increased bone-turnover such as bone infections. However, in subsequent clinical studies where ^{224}Ra is used to treat bone tuberculosis even extremely high doses were not effective and over time, led to a significant increase of bone tumors.[43] This suggests that a more selective targeting is necessary to utilize the full potential of these alpha- and beta-emitting radionuclides. Dadachova showed that using antibodies as a transport vehicle for delivery of radioisotopes, just like radioimmunotherapy used in the field of oncology, bacteria and fungi can be targeted with high specificity. RIT relies on the antigen-binding characteristics of the antibodies to deliver cytotoxic radiation to target cells. As microbes express antigens that are unique and different from host antigens, they can be targeted with high specificity and low cross-reactivity. It could especially be of great

value in biofilm related infections where dormant cells are metabolic inactive and therefore not susceptible to most antibiotics because the damaging effects of radiation are independent of the cell's metabolic state. To improve RIT further, smaller vehicles can be used such as nanobodies. These nanobodies are derived from camelids and are ten times smaller than conventional antibodies. Due to its size, nanobodies have increased elimination to get rid of the potential dangerous remaining unbound radioimmunoconjugates minimizing collateral damage even further. Also, they have considerable better penetration into tissue and presumably the biofilm. Other advantages include high stability, solubility, expression, and specificity. Theoretically, a patient with a periprosthetic joint infection where the hip implant is colonized with bacteria and a biofilm, could be treated with nanobodies labeled with an alpha-emitter like ^{213}Bi or ^{225}Ac that can penetrate deep in the biofilm, destroy the architecture and kill bacteria. (Figure 3) These antibodies could also be a powerful diagnostic tool for positron emission tomography (PET)-imaging when labeled with positron-emitting radioisotopes such as fluor-18 (^{18}F) or zirconium-89 (^{89}Zr). Due to the high specificity and rapid clearance, low background signal is expected so that even low-grade infections could be detected with high specificity and sensitivity.

Treatment and diagnostics with radiation is always prone to safety concerns. Alpha- and beta-emitting radioisotopes such as ^{223}Ra and ^{188}Re are already used in the clinic for metastatic castration-resistant prostate cancer. Safety studies show that treatment with these radioisotopes is associated with minimal adverse events.[60,61] Nonetheless, it is important to consider survival time, age, physical and emotional wellbeing and alternative treatment options. As the 5-year survival of PJI patients is lower than the predicted survival for melanoma, prostate and breast cancer, aggressive treatments seem justified. Sometimes, infection surgery yields great risk to the point that only lifetime antibiotics or amputation is an option. Further development of antibiotic resistance due to antibiotic treatment reduces the chance of successful treatment even

further. In these cases radiation treatment could be beneficial despite the possible long-term effects although these risks may be limited.

Conclusion

The need for alternative treatment options for patients with (implant) infections like periprosthetic joint infections grows every year, not only due to increasing pathogen resistance to antibiotics, but also because biofilm formation obstructs the treatment of these infections with antibiotics. The novel prospects of radiation treatment strategies against planktonic and biofilm-related microbial infections are worth to investigate further.

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Figure 1: Hand mit Ringen ("Hand with rings"), the first medical radiograph of the left hand of Wilhelm Röntgen's wife, Anna Bertha Ludwig taken more than a century ago on December 22, 1895. (The picture is in public domain)

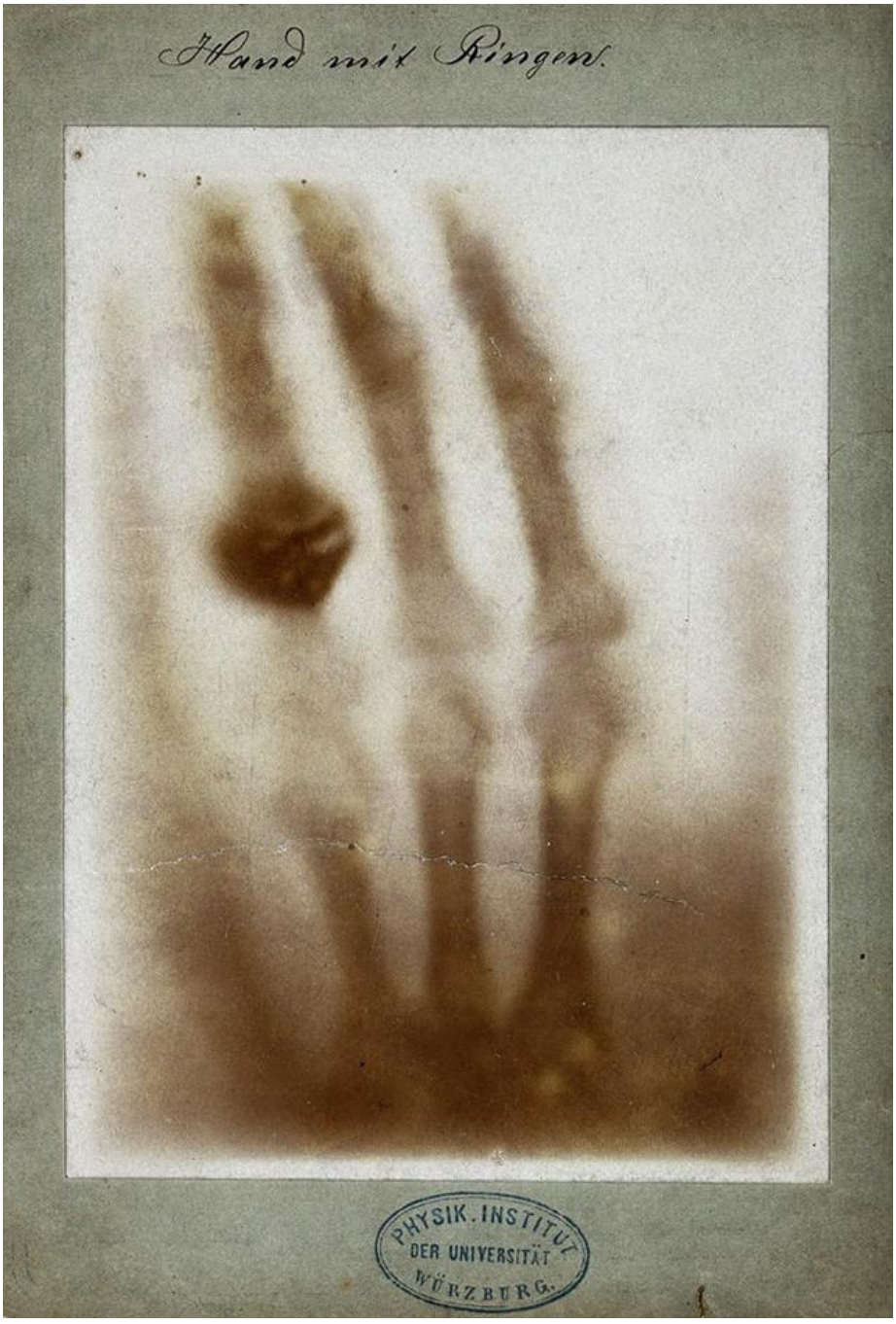

Figure 2: Flowchart of systematic search and methodology
Summarizes the screening process resulting in 170 publications included in this review.

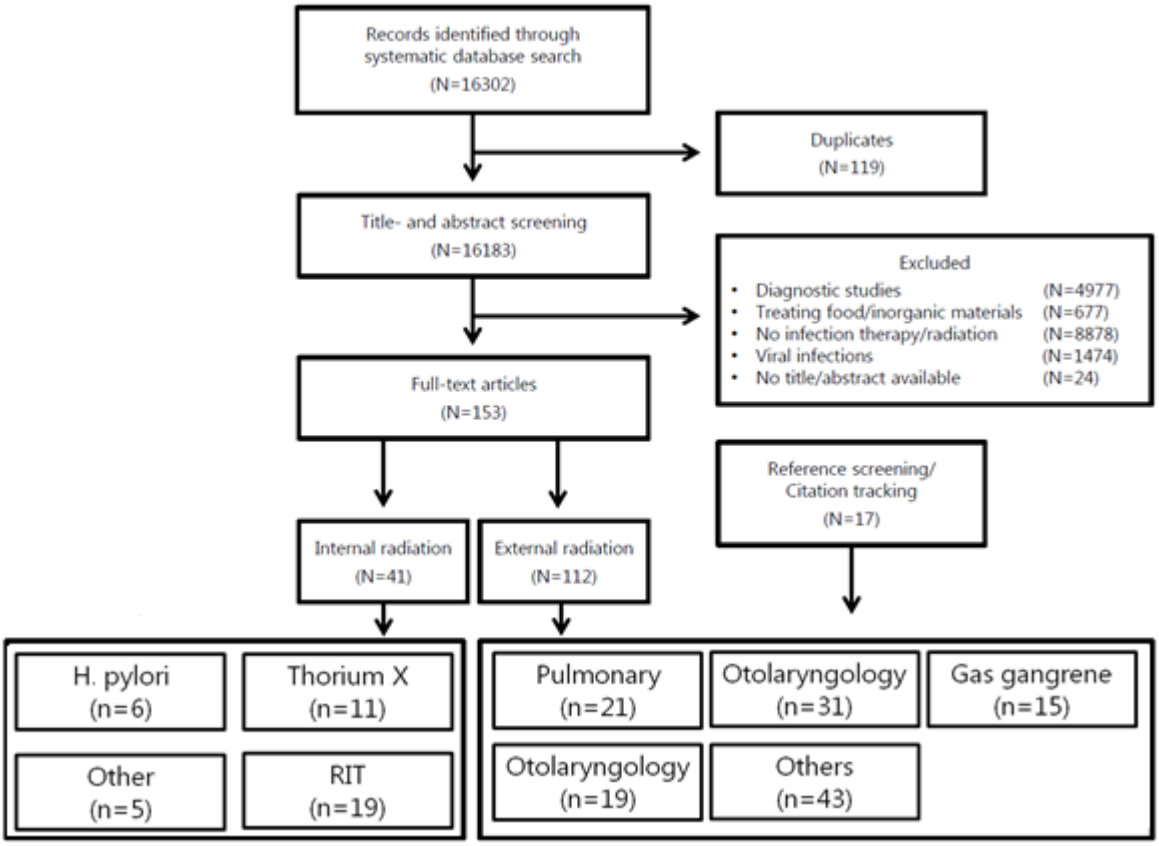

Table 3: Eligibility Criteria

External Radiation	
Inclusion Criteria	**Exclusion Criteria**
Investigates treatment of bacterial or fungal infection with radiation	Diagnostic studies
Human, clinical study	Indirect use of radiation
	In vitro research
	No abstract/full-text available
	No English/German/Dutch language
Internal Radiation	
Inclusion Criteria	**Exclusion Criteria**
Investigates treatment of bacterial or fungal infection with radiation	Diagnostic studies
	No abstract/full-text available
	No English/German/Dutch language

Video 1: The follow-up of the radium applicator incident in the Dutch city of Putten

[Transcript: The small town of Putten in the area called "De Veluwe" has gained worldwide attention, because the house of the Haanschoten family in the "Schoolstraat" was contaminated with radioactive material after a medical treatment. Quickly following the discovery, the immediate surroundings of the house were isolated. The garden, in which radioactive ashes were sprinkled, was covered with a plastic tarp, to prevent contaminated dust being blown away by the wind. All inhabitants of the town that had been in contact with the Haanschoten family were medically examined. They had to go to the police headquarters in Putten. At the police station they were investigated with devices that could detect the presence of radioactivity. Luckily, nobody was found to be contaminated during the investigation. Among them, the friend of the 5 year old Joke Haanschoten, who had to leave her house in Putten and who had to be admitted and observed at the hospital in Utrecht, together with her parents, brother and sisters.]

Source: Dutch Institute for Image and Sound, https://eye.openbeelden.nl/media/665796

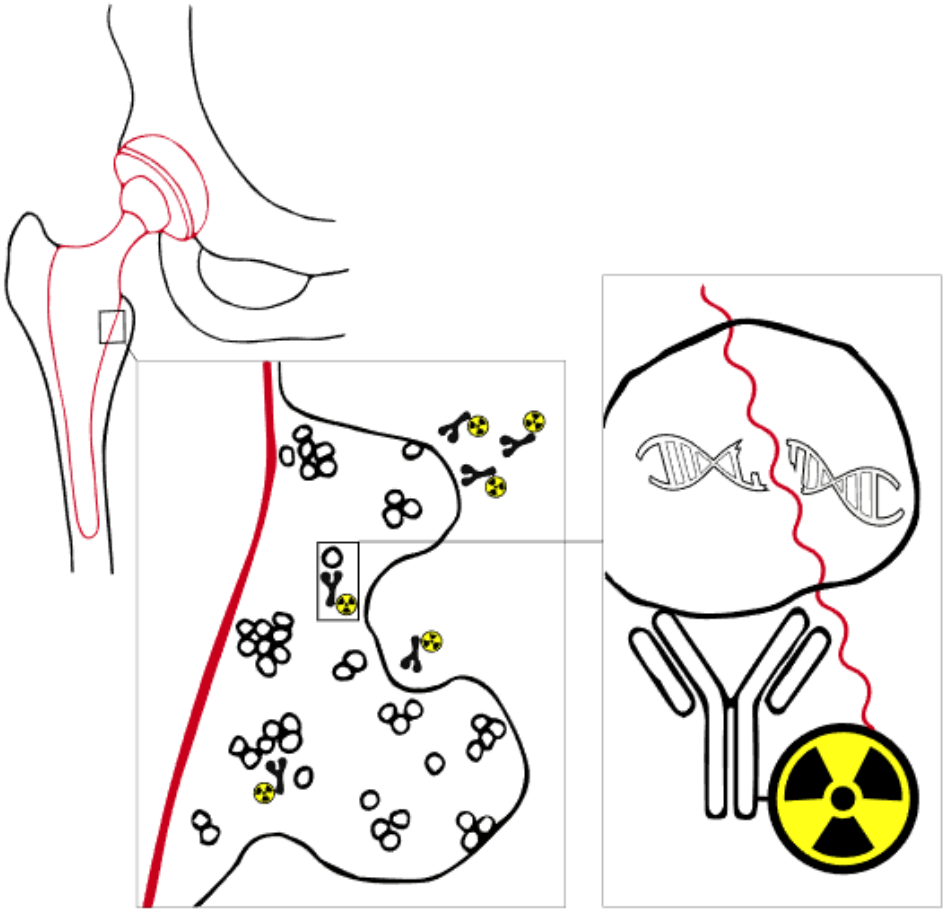

Figure 3. Concept: Radioimmunotherapy for periprosthetic join infections. Bacteria form a biofilm on the hip prosthesis that protects them from antibiotics and the immune system. Targeted radiation therapy with alpha- or beta-emitting radioisotopes could be able to destroy the structure of the biofilm and kill the bacteria.