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The dream of near-zero x-rays ablation comes true

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Abstract:

While radiation exposure related to natural sources plays a minor role, medicine-related exposure, represents, to date, a major exposure source. Within this exposure interventional electrophysiology is a relevant contributor. Unfortunately no safe dose in radioprotection exists, the negative acute and long term effects of radiological exposure may emerge at any radiation exposure dose. For this reason patients and physicians should be aware of the risk of radiation exposure and the benefits of the imaging/procedure balanced by the required radiation exposure. Given this, performing a near to zero x-rays transcatheter ablation procedure should therefore represent an aim for all electrophysiological lab. Fortunately, the introduction of electroanatomic mapping systems, have provided the possibility to perform simple and complex electrophysiological procedures avoiding, or at least, limiting the use of radiations. The present review summarizes state of the art of feasibility and safety of the near to zero approach for the main electrophysiological procedures, highlighting the potential health benefits.

Introduction

Over the last two decades, the field of cardiac electrophysiology has undergone tremendous change and evolution. The scope of available therapies has widened considerably, and hitherto ñuntreatableò arrhythmias have become commonplace in most laboratories. More complex arrhythmia substrates such as atrial fibrillation (AF), atypical atrial flutter (FL), and ventricular tachycardia (VT) are no longer relegated to medical therapy, however these more extensive ablative procedures result in longer procedural times, and consequently, longer fluoroscopy times. Given that considerable evidence exists as to hazards of this exposure to both the patient and the medical personnel, developing technologies and techniques to reduce fluoroscopy use in the electrophysiology environment is crucial[1].

The first step to reduce fluoroscopy exposure is to optimize setting and use of the fluoroscopy system. It is, in fact, demonstrated that parameters such as pulse rate, collimation, detector sensitivity/distance, and signal filtering significantly impact radiation exposure[2]. On the other hand, the advent of three-dimensional electroanatomic mapping (3D-EAM) and cartographic systems, originally introduced to allow electrophysiologists to target more challenging arrhythmias by offering activation/voltage data and visualization of the catheters and of the created lesions in 3D views, have further helped to reduce fluoroscopy exposure, advancing our field towards the dream of near-zero radiation interventions.

Biological effects of radiation and evidence of harm.

Radiation exposure related to natural sources is relatively minimal, whereas nowadays, medicine-related exposure is considered a major exposure source. Hydroxyl radicals created by X ray interaction with water molecules interact with the DNA causing strand breaks or base damage. In addition X-rays also ionize DNA directly. Cellular clearance and repair mechanisms limit oxidative damages to the DNA. However, when double strand damages rather than point mutations occur, radiation related injury can be caused. In general, radiation injuries are induced by the stochastic mechanism or the deterministic effect. The stochastic mechanism represents an unpredictable, unrepaired radiation damage to the DNA of a limited, potentially single, number of viable cells. The deterministic effect, instead, occurs when a significant, predictable number of existing cells are sufficiently damaged to cause a directly observable injury. Radiological induced damage can be evident following both acute exposition (eg radiation induced skin injuries) or during long term follow up. In medicine, both radiological examinations (eg. computed tomography, and scintigraphy scans) and several interventional procedures (eg. percutaneous coronary intervention, arrhythmia ablation) may expose to a significant amount of radiation. In fact, data from large sample size observational studies reported an increased malignancy rate, over a long term follow up, both for patients undergoing radiological examinations than electrophysiological procedures[3]. An intervention such as AF ablation, for example, exposes patients to a dose of about 15 mSv (ranging from 1-60 mSv), increasing the absolute lifetime risk of a fatal cancer in an adult by 0.08% (considered on a background fatal cancer risk of about 20%)[4] and this biological harm is obviously even higher within the paediatric population[5]. Additionally, the exposure risk is relevant not only for the patient but also for physicians and lab personnel[6]. It has been estimated that an interventional cardiologists presents a median radiation exposure per year equivalent to approximately 250 chest X-rays (5mSv), a two to three times higher dose compared to a typical radiologists, and this has recently been related to an increased risk of cognitive impairment and brain malignancy (in particular on the left side) [7,8,9]. For these reasons, in the attempt to avoid an uncontrolled increase in radiation exposure and consequent risks, the American College of Cardiology stated that the risk of radiation exposure always needs to be balanced by the benefits of the imaging/procedure itself. Based on the following three principles: justification, optimization, and responsibility for dose limitation, all interventional laboratories should therefore mandatory be guided by the óALARAô (radiation doses óAs Low As Reasonably Achievableõ) principles[10].

3D mapping systems in Electrophysiology

Electrophysiological procedures have originally been performed by fluoroscopic guidance. 3D-EAM systems have emerged as an alternative with the potential to limit or, in some case, avoid radiation exposure. To date, four 3D-EAM systems are widely used to visualize electrophysiology catheters without X-rays (Table 1): EnSite NavX and Mediguide technologies (both by St. Jude Medical, St Paul, Minnesota, USA), Carto (Biosense Webster, Diamond Bar, California, USA), and Rhythmia (Boston Scientific San Jose, CA. 95134 USA).

The EnSite NavX relies on three pairs of nominally orthogonal skin patches in x-y and z-axis positioned on the patientôs chest. These patches create an electrical location field on the patients thorax. An additional patch positioned on the abdomen serves as a reference during advancement of the catheters in the iliofemoral venous axis. The system collects electrical data from standard electrophysiology catheters and uses this information to track or navigate their movement, construct 3D models of the chamber and create activation and voltage maps. Some years later, the same company, has also introduced Mediguide technology. This system provides the possibility to move catheters into previously acquired fluoroscopic loops. Pre-acquired ecg and respiration triggered biplane short sequences of conventional fluoroscopic frames allow traditional catheter and structure visualisation tracking within dynamic, virtual cardiac chamber models (4D model) [11]. Catheter positioning system is based on a dynamic electromagnetic field integrated with a miniaturized single coil sensor mounted on dedicated electrophysiology catheters and a reference sensor attached to the patientôs chest[12]. It should however be reminded that pre-acquired fluoroscopy loops only provide an illusion of real-time imaging. Acute clinical variations, such as a pericardial tamponade or a pneumothorax, will not be real-time depicted.

The Carto system, in its present third generation (Carto 3), instead, is based on six skin patches positioned on patientôs chest and back that create an electrical based location field. These patches, by a location pad technology (with 9 coils), also create a magnetic field. The combination is, therefore, an electro-magnetic field in which catheter movements can be detected. As the catheter moves around the chamber, a multitude of such associated locations are created and stored by the system. Advanced Catheter Location Technology combines the magnetic location technology with current based visualization data in a virtual chamber reference system built by catheter movements. As with most of the other systems, this technology offers the possibility of merging the virtual chamber with a pre-acquired anatomical image (e.g. magnetic resonance (MRI) or computed tomography) allowing physicians to navigate catheters in an accurate representation of the patientôs anatomy. Also with this system, activation mapping information, during arrhythmias or sinus rhythm, may be projected to the map with a color coded mode, useful for guiding the ablation to the origin of the arryhthmia. The Carto-Univu, a module permitting real-time catheter tracking superimposed on pre-recorded cine loops, has further implemented the potential of this system. Recently a new 3D-EAM, Rhythmia, based on both magnetic and impedance 1-2 mm accuracy localization, has been introduced. This system is based on an open architecture permitting the choice of different diagnostic catheters. However, activation and substrate mapping can be performed only with a dedicated catheter (IntellaMap OrionÊ) that has the peculiarity of being a basket, high resolution mapping catheter with 64 low-noise electrodes and 2.5 mm inter-electrode spacing. Thanks to the latter and an advanced point acquisition software and process, this system is able to generate, by automated and continuous mapping, accurate, high-resolution 3D electroanatomical maps.

In addition, driven by awareness of the beneficial effects of acquiring anatomical details by limiting fluoroscopy exposure, new attractive systems continue to become commercially available. For example, the recently implemented AcQMap system,[13] combining ultrasound guided anatomical details to high density bipolar or unipolar voltage signals obtained from a hybrid catheter with 48 ultrasound transducers and 48 electrodes, seems indeed promising. Eventually, having cardiac MRI the capability to show both anatomic and functional tissue data without ionizing radiation, growing interest is directed towards the possibility of real time MRI catheter tracking, obviously, in this case, not only in the electrophysiological field[14].

Near zero fluoroscopy approach.

The following is an overview of the state of art on the use of the aforementioned technologies for the most frequently encountered electrophysiological procedures, specifically focusing on feasibility, safety and radiation exposure. Available literature has been critically analysed in the attempt to provide an independent point of view. However, given that the majority of the technologies described are available and in current use at our centre, a personal interpretation, deriving from personal clinical experience, may emerge. In any case, it should be reminded, that none of the commercially available 3D-EAM systems provide such a real-time and location precision compared to traditional fluoroscopy. In fact, in addition to what stated above on the limits of pre-acquired fluoroscopy loops providing only an illusion of real-time imaging, electroanatomic systems deeply rely on reference stability (e.g. electrical signals/external patches/electromagnetic field), accurate registration, and patientǿs immobility. Moving catheters into sensitive regions (e.g. left main or right coronary artery during a retrograde aortic approach), should, in case of uncertainty, always be guided by real-time fluoroscopy. On the other side, fluoroscopy, providing grey scale frames in which the physician recognizes the different anatomic structures based on shadows, position in the chest, appearance and catheterǿs movement/signal, strongly relates on the physicianǿs experience.

Typical atrial flutter Typical FL is an atrial arrhythmia maintained by an anatomical re-entry localized in the right atrium. In this arrhythmia, the portion between the inferior vena cava (IVC) and the annulus of the tricuspid valve is the critical isthmus and the target of the percutaneous ablation procedure. Due to the unsatisfactory success rates of antiarrhythmic drugs, to date, percutaneous ablation of typical FL represents the first line approach[15]. Given the relatively simple anatomic positioning of catheters to treat this arrhythmia, near zero fluoroscopy approaches for typical FL ablation have been reported since more than ten years[16, 17, 18,19]. The clear evidence emerging is that 3D-EAM systems broadly reduced fluoroscopy exposure without affecting procedure safety and outcome. In a recent study by Macias et al [20], a zero fluoroscopy approach was attempted in all consecutive procedures of typical atrial flutter. In this series, a duo-decapolar catheter and an irrigated-tip ablation catheter were inserted via two punctures in the femoral vein and fluoroscopy was to be used only in case of challenging catheter positioning. In over 60 cases, no fluoroscopy was used for about 90% of the procedures. Even more recently Schoene et al [21] systematically applied Mediguide technology to 20 patients undergoing percutaneous ablation of cavo-tricuspid isthmus reporting no difference both in terms of freedom from recurrences, safety, and procedure duration while achieving a significant radiation exposure reduction.

*Atrioventricular nodal re-entrant tachycardia***.** Atrioventricular nodal reentrant tachycardia (AVNRT) is perhaps the most frequently encountered supraventricular tachycardia (SVT) in the electrophysiology lab. Given the high success and low complication rates, transcatheter ablation is considered first line therapy[22]. Since it may be performed in centres with differing levels of experience, fluoroscopy times for this procedure are quite variable. However, commonly involving children or women in child-bearing age, the potential for radiation exposure is non-negligible. Kopelman et al initially reported on the use of nonfluoroscopic mapping systems for a common AVNRT [23]. As would be intuitively expected, fluoroscopy times were significantly decreased when compared to conventional approach (4.2+/-1.4 vs 15.9+/-6.4 min). Importantly, this did not impact negatively on success and complication rates and total intervention times. Evidently, interest for this in the paediatric population led to similar reports confirming diminished fluoroscopy times[24]. The feasibility of eliminating x-ray use altogether by using a 3D-EAM system was demonstrated in a relatively small paediatric study showing a 95% reduction in fluoroscopy time with 24 of 30 patients requiring no fluoroscopy whatsoever [25]. The ñnear-zeroò radiation objective has been achieved in subsequent studies using the NavX system for all steps of the procedure instead of fluoroscopy, other than in a variety of arrhythmias, also for AVNRT [26,27] (Figure 1).

In fact, the most recent innovation of the systems permitting real-time catheter tracking superimposed on pre-recorded cine loops, as Mediguide (St Jude Medical) and Carto-Univu (Biosense Webster) have facilitated this approach also in physicians fond of traditional fluoroscopic views[28,29].

Atrioventricular re-entrant tachycardia. Reciprocating atrioventricular reentry tachycardia (AVRT) is a SVT maintained by the presence of an accessory pathway (AP, Figure 2). Given the typical young age of patients involved and the high efficacy of the treatment, radiofrequency ablation is here again considered the first line option. Conversely, for the same reason, radiation exposure due to an interventional approach in these patients has been of concern. Drago et al [30] reported his experience on 22 paediatric patients in whom a non-fluoroscopic approach was attempted using the Carto mapping system. In this series, ablation success rate was 95% and no complications occurred. A more recent study reported on the systematic use of a non-fluoroscopic approach in a larger cohort of 328 patients[31]. This group consisted of 35 patients with AVRT whereas patients with left sided APs or those who needed a transeptal puncture were excluded from the study. Procedural success was achieved in 99.1% of cases and in 94.7% the procedure was completed without any fluoroscopy use at all. A wider experience has been recently reported by Scaglione et al[32]. In this series a total no fluoroscopic approach was used in 44 consecutive paediatric patients with planned AP ablation. Right chambers were accessed through a venous transfemoral approach while a retrograde transaortic approach was used to access mitral annulus. Only three cases of left sided APs were ablated through a patent foramen ovalis. In this experience a total of 47 AP (left sided 45%) were ablated without the use of fluoroscopic guidance. Eventually, a multicentre, randomized, controlled experience has recently become available in this group of patients[33]. In this experience on 262 patients undergoing electrophysiological study for SVT, 72% of the AVRT procedures were performed without any use of fluoroscopy. Unfortunately, in this setting, left sided AP requiring transeptal puncture were excluded. As discussed later in the AF ablation section, intracardiac echography (ICE) may eventually play a role in reducing fluoroscopy exposure during transeptal puncture[34] also for left sided AP, but such an approach still needs to be standardized and validated.

Ventricular tachycardia Percutaneous ablation of VT has been increasingly recommended (Figure 3). VT may be triggered by an ectopic focus or, especially in case of an underlying cardiomyopathy, by an area of slow conduction (eg. scar due to a previous myocardial ischemia) localized in the myocardial muscle mass, creating the ideal substrate for re-entry triggered arrhythmias. In any case, the ablation procedure necessitates identification of the ectopic spot and/or the critical isthmus of the arrhythmia. For both right and left sided VT, 3D-EAM systems have the potential to significantly reduce fluoroscopic exposure. In addition to this, the ability to perform substrate mapping (based on both voltage and activation maps) in sinus rhythm together with the detection of fractioned or late potentials (seen in the scar areas in underlying ischemic heart disease) hold the great advantage of identifying ablation targets without inducing and/or maintaining clinical arrhythmia (commonly not well tolerated)[35]. The first study looking at the feasibility of a near to zero approach to the ventricles has addressed right ventricular outflow tract (RVOT) premature ventricular contractions (PVCs) both in adults and in the pediatric population[36]. More recently 3D-EAM proved feasible also for treating PVCs in adult patients with complex congenital heart disease[37] and even, by the use of the CartoUnivu system in a high volume center, for ablation of VTs by epicardial approach (without increasing complications rates)[38].

Overall it is becoming clear that, in this setting, activation mapping during VT together with substrate mapping enhance the clinical utility of 3D-EAM systems beyond radiation exposure reduction. However, complex and/or left-sided VTs not amenable to a retrograde approach remain, for the moment, best suited to fluoroscopic guidance.

Atrial fibrillation AF is the most common arrhythmia in the adult population (particularly in older patients), and it represents the widest indication for transcatheter ablation. In fact, considering the limited efficacy of antiarrhythmic therapy, often associated with relevant side effects, percutaneous ablation has emerged in recent years as the perhaps most promising therapeutic strategy. Two main mechanisms are involved in AF onset: triggers and perpetuation. Arrhythmia triggers are commonly localized in the pulmonary veins and this mechanism of arrhythmia is particularly relevant in young patients without underlying cardiomyopathy (and atrial remodelling) suffering paroxysmal AF. Perpetuating factors, instead, are related to atrial enlargement, tissue fibrosis and consequent shortening of atrial refractory period. This mechanism is particularly observable within patients with underlying cardiomyopathy suffering persistent or long-standing AF.

3D-EAM systems able to evaluate instantaneous catheter positioning, respiration triggered movement and offering the possibility of integrating radiological images are allowing users to perform pulmonary vein isolation (Figure 4) and left atrial substrate modification with minimal use of fluoroscopy[39],[40]. In addition to 3D-EAM systems, other technologies have emerged to facilitate AF ablation: contact force technology, for example, is able to monitor and measure the tissue/catheter contact in order to avoid excessive or insufficient forces on the tip of the catheter. After a brief learning curve, this technology further supports manoeuvring in a zero fluoroscopy setting[41],[42]. In a multicentre study on 240 consecutive patients undergoing catheter ablation of AF, adoption of a 3D mapping system proved to significantly impact routine activity in all centres involved, achieving an average fluoroscopy time decrease from 26 ± 15 min to 16 ± 12 min ($P < 0.001$)[43]. More recently a prospective, randomized, blinded trial, clearly showed that the systematic use of third generation EAM systems reduced fluoroscopy exposure in patients undergoing AF ablation, without increasing procedure duration or affecting safety and short-term efficacy [44]. Eventually the systematic use of EAM systems integrated with preacquired imaging, in this case cardiac magnetic resonance with use of oral gadobenate dimeglumine,[45] also presents the advantage of visualizing the esophagus, potentially limiting occurrence of atrio-esophageal fistulas, a rare but potentially fatal periprocedural complication.

As previously stated, to date, the only remaining phase that limits a complete zero fluoroscopy approach for AF ablation, and other left sided arrhythmias not approachable by retrograde aortic access, is transeptal puncture (TS). In this respect, preliminary data suggest that ICE may become a routine strategy to guide TS without fluoroscopy use [39], [40]. A recent experience in 80 patients showed that by using a third generation mapping system, contact force technology and ICE guiding TS, RF ablation of AF was not only feasible without fluoroscopy but also safe, without affecting procedure duration, radiofrequency application time and mid-term efficacy [46]. Mansour et al [47] recently reported on the use of the equipment compatible with the Mediguide Technology in order to perform a TS without fluoroscopy in a small population of consecutive patients. In fact, by the use of a guidewire with a magnetic sensor on the tip, the authors managed to perform the TS with a very low fluoroscopy exposure.

Reasons for performing a fluoroscopy-free procedure

How is radiation exposure risk perceived by the patient? How many patients would decline undergoing a diagnostic imaging scan or an interventional procedure to avoid increasing their lifetime cancer risk? To date, awareness on the topic is poor. In our opinion patients need to be sensitized to radiation exposure risks. How is radiation exposure risk perceived by physicians? Unfortunately, although long-term effects of radiation exposure are relevant, awareness is suboptimal in physicians as well.

Though extremely simplified, to provide a significant highlight on the expected results of an increased awareness on the topic, we suggest circulating the findings of the recent NO-PARTY Trial[33]. This study, has reported a potential 96% reduction in the estimated risks of cancer incidence and mortality and a significant reduction in estimated years of life lost and of life affected by a zero fluoroscopy approach compared to the conventional approach. A conventional procedure in a 35 years old patient will result in 1 week of ñlife lostò and 2 weeks of ñlife affectedò in contrast to 5 and 12 hours, respectively, by performing a fluoroless procedure. Other compelling risk reductions are summarized in Figure 5.

On the other side the near to zero fluoroscopy approach should not increase other risks. For this reason available data focusing on safety of the approach, based on limited sample sizes and only few randomized studies, cannot, to date, be considered conclusive.

Future perspective. Biological effects of radiation are known and, in our opinion, respecting the ALARA principles should be mandatory to respect the health of both operators and patients. In the last two decades, technology has significantly improved, and we can currently use the systems and techniques hereby described to achieve relevant radiation exposure reductions, making the ñnear to zero fluoroscopy approachò indeed feasible, without affecting safety, efficacy, and procedure duration. On the other hand the central role of physicianôs education needs to be outlined. A low fluoroscopic procedure depends upon technology but also on a thorough, regulated and controlled training and teaching of the physicians. This is of paramount importance, especially in low volume centres with, perhaps, little experience and training on the most recent systems.

The medical community and patients need to become aware of this possibility to permit and support the evolution from traditional electrophysiology practices towards approaches that significantly limit the use of fluoroscopy. The only significant issue that remains is how to minimize fluoroscopy during the TS for left-sided procedures, and achieving this could potentially nearly eliminate radiation use. Presently, the usual anatomic landmarks used to guide TS can be identified on fluoroscopy but not by 3D-EAM systems. The use of other technologies such as ICE, transoesophageal echocardiography or sensor enabled guidewires are still relatively cumbersome, requiring adjunctive vascular access, deep sedation, and/or extra intravascular material. Once the transeptal needle becomes traceable in any of the available non-fluoroscopic mapping systems, a total non-fluoroscopic approach will be possible for the vast majorities of electrophysiological procedures.

In conclusion, motivated by evidence of feasibility and of a clear measurable cancer risk reduction, all electrophysiology laboratories should aim to significantly reduce X-ray exposure by the judicious of available systems and technology. Meanwhile, continued technological progress is clearly showing that the dream of a complete zero fluoroscopy approach for all arrhythmias management is close at hand.

Table 1. Available electroanatomic mapping software
Different electroanatomic mapping software to date available to perform minimally fluoroscopic exposure procedures.

Commercial name	Ensite NavX	Mediguide Technology	Carto 3 system	Localisa	Rhythmia
Localization based system	Voltage guided field	Low powered electromagnetic field	Magnetic and Impedance field	Electrical field	Magnetic and Impedance field
Movement sensibility (mm)	1.4	0.5	1.0	1.4	1-2
Multipoint mapping catheter available	Yes (max 128 point)	Yes (max 10 point)	Yes (max 20 point)	No	Yes (max 64 point)
Open architecture system	Yes	No	Yes	Yes	Yes
Possibility to merge with preacquired images	Yes	Yes	Yes	No	Yes

Figure legends

Figure 1. Cryoablation of a slow pathway potential (white arrow) by EnSite NavX (left anterior, oblique and right anterior oblique views). In white, tetrapolar hissian catheter (Supreme CRD2 5F, St Jude Medical). In green, decapolar coronary sinus catheter (Inquiry 6F, St Jude Medical). In blue, tetrapolar for right ventricle catheter (Spike Ultra 6 F, FIAB). In Yellow, tetrapolar ablation catheter (CrioCath Freezor Xtra, 8F, Medtronic).

Figure 2. Left side accessory pathway ablation by EnSite NavX and Mediguide systems. In green, decapolar coronary sinus catheter (Inquiry 6F, St Jude Medical). In blue, tetrapolar for right ventricle catheter (Spike Ultra 6 F, FIAB). In Yellow, tetrapolar ablation catheter (Therapy Cool Path Duo 8F, St Jude Medical). Ablation catheter positioning at the left portion of the mitral annulus is guided by both the EnSite NavX left lateral view (left) than by visualization (yellow tip) in the pre-acquired left lateral fluoroscopy loop (right) in the Mediguide system.

Figure 3. Right ventricle outflow tract tachycardia ablation by Carto 3 system (left). Activation map during tachycardia (left lateral view) localizes the earliest activation site (red) at the posterior portion of the right ventricle outflow. Radiofrequency delivery at this site (red dots) eliminated the arrhythmia. Left ventricle ventricular tachycardia ablation (right) in a patient with coronary artery disease. Voltage map documented a wide scar area (<0.5 mV) at the apex of the left ventricle. Activation map during ventricular tachycardia (antero-cranial view) showed earliest activation (red) at the anterior portion of the periscar area (in presence of a mid-diastolic fragmented potential). Please note contact force technology (Smart Touch catheter, Biosense Webster) advising of a 3/9 gr pressure on the ventricular wall.

Figure 4. Atrial Fibrillation ablation by Carto 3 Univu system. Left: anatomic reconstruction (grey) of the left atrium (performed by circular decapolar Lasso Catheter decapolar 7F, Biosense Webster) before merging to the pre-acquired MR scan (yellow). Center: posterior and left lateral Carto views showing ablation points (red) obtaining pulmonary vein isolation (Navistar Smart Touch 8F, Biosense Webster). Right: antero-posterior and postero-anterior views of the left atriumôs anatomical map integrated in the fluoroscopic background (Carto Univu module; Ez steer decapolar coronary sinus catheter 7F, Biosense Webster).

Figure 5. Lifetime Attributable Risks of cancer mortality with 95% confidence intervals from minimal fluoroscopic approach (N=118) and conventional approach (N=113) ablations in function of age at exposure and sex (number of cases in 100.000). Data From Casella M et al Europace 2015.

Figure 1

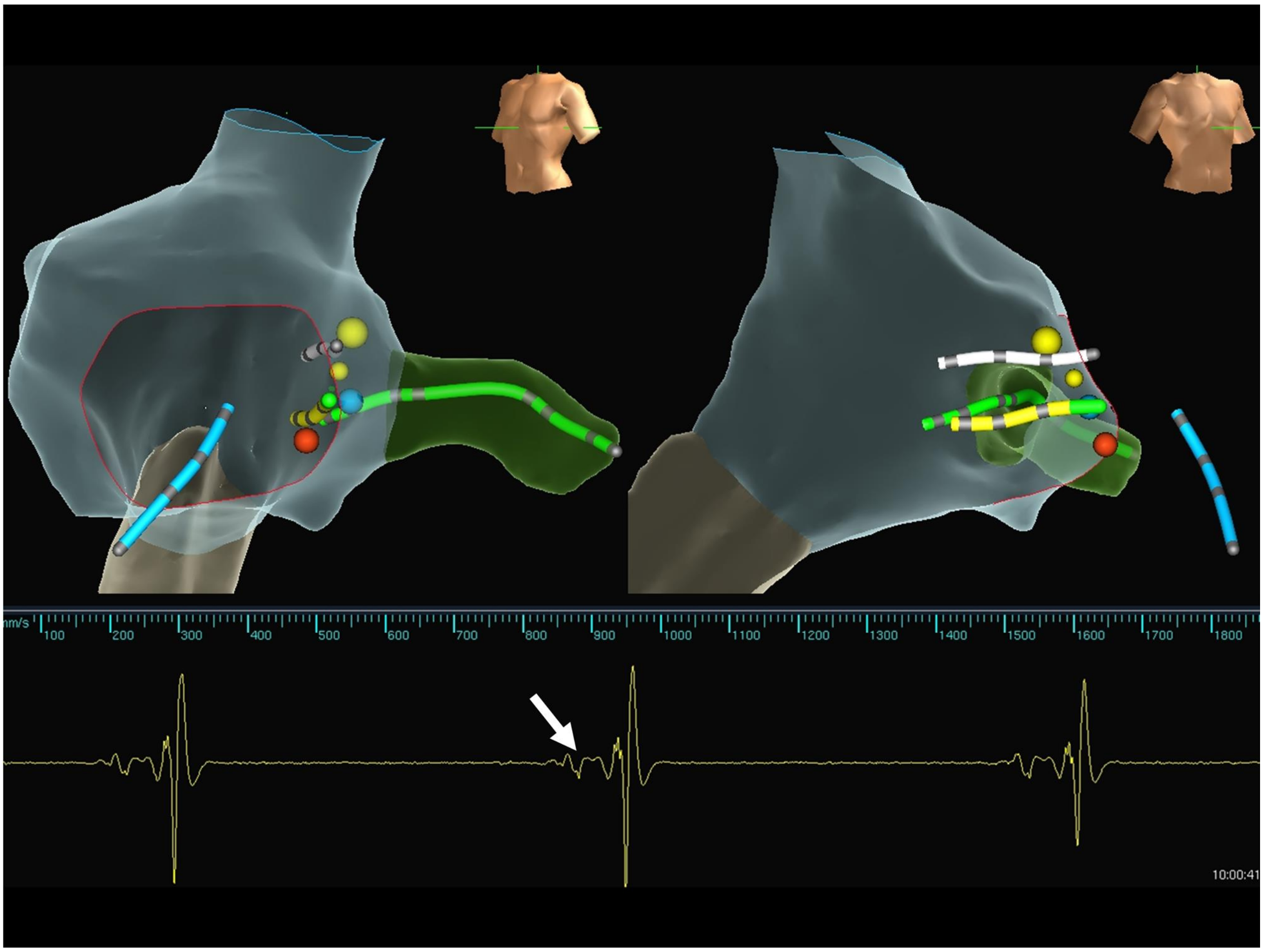
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Figure 2

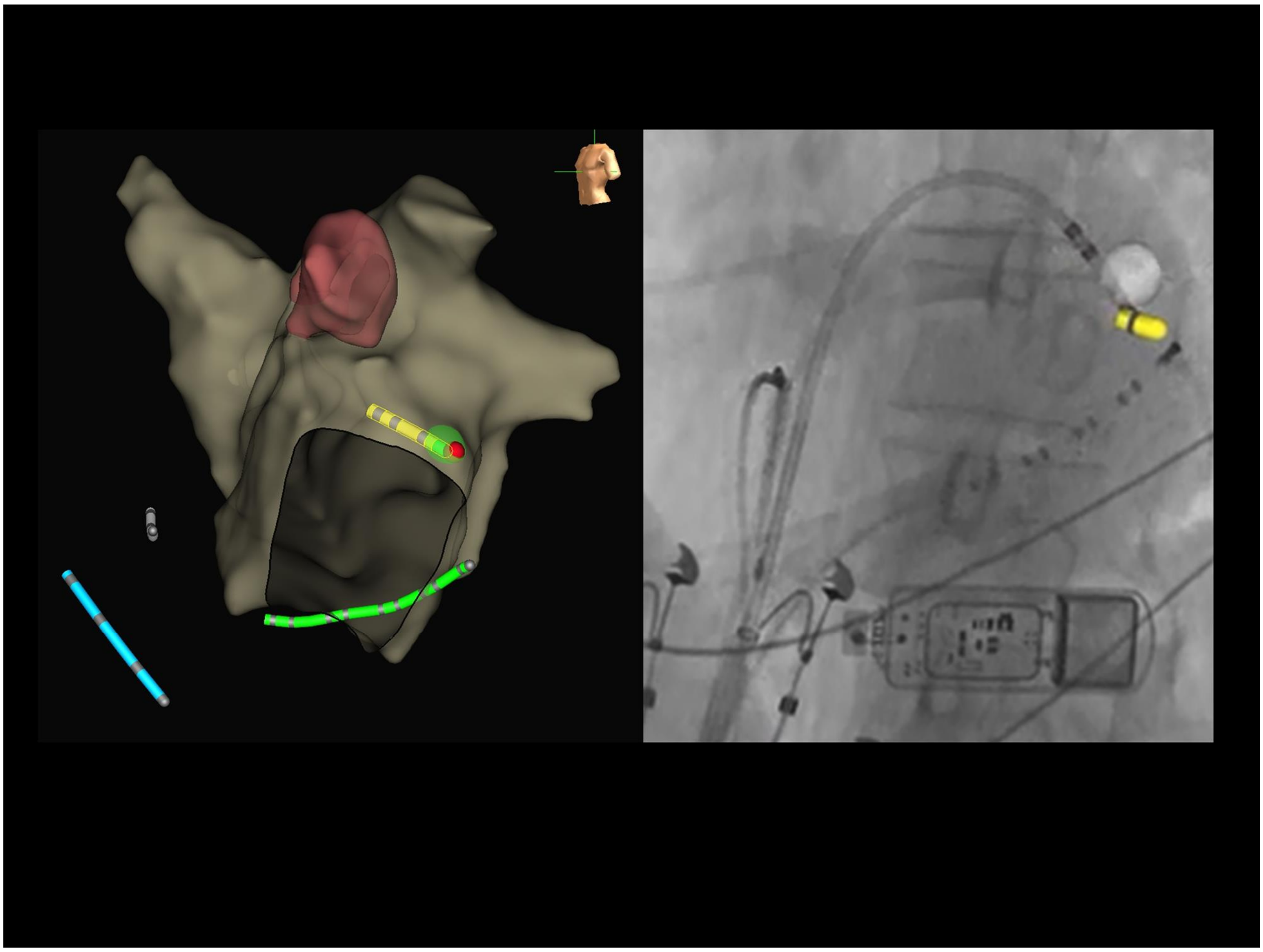


Figure 3

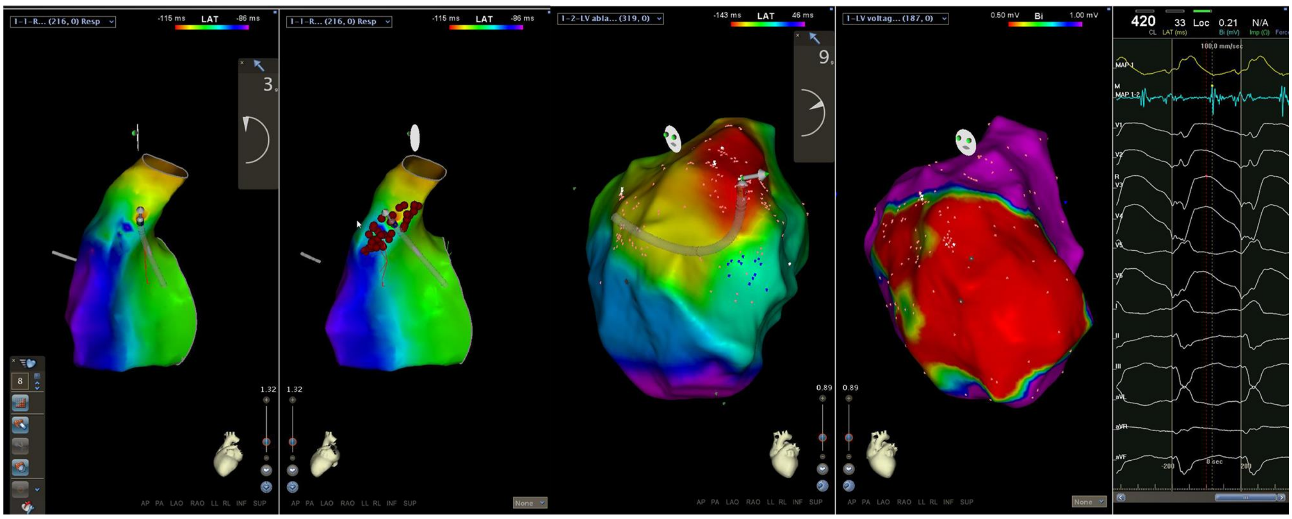


Figure 4

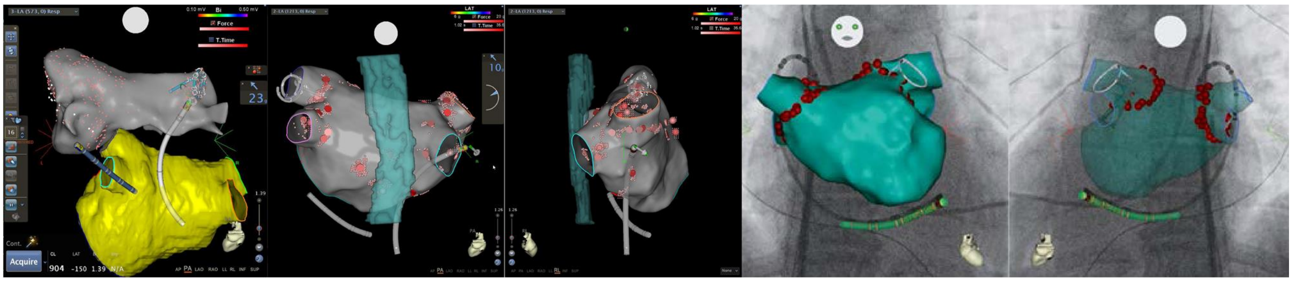
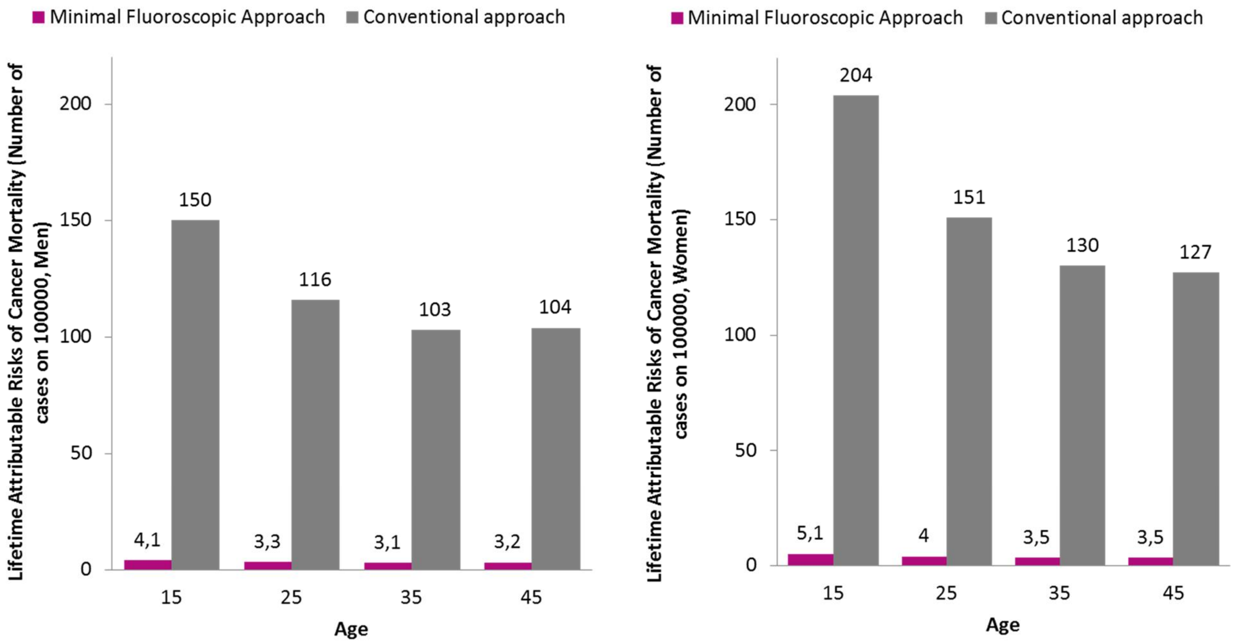


Figure 5



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