Sentinel lymph nodes fluorescence detection and imaging using Patent Blue V bound to human serum albumin

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Abstract: Patent Blue V (PBV), a dye used clinically for sentinel lymph node detection, was mixed with human serum albumin (HSA). After binding to HSA, the fluorescence quantum yield increased from $5 \times 10^{-4}$ to $1.7 \times 10^{-2}$, which was enough to allow fluorescence detection and imaging of its distribution. A detection threshold, evaluated in scattering test objects, lower than $2.5 \text{ nmol} \times \text{L}^{-1}$ was obtained, using a single-probe setup with a 5-mW incident light power. The detection sensitivity using a fluorescence imaging device was in the µmol × L^{-1} range, with a noncooled CCD camera. Preclinical evaluation was performed on a rat model and permitted to observe inflamed nodes on all animals.

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1. Introduction

The sentinel lymph node (SLN) biopsy is a minimally invasive surgical procedure used worldwide to evaluate lymph node status in early breast cancer [1,2]. The SLNs are the first nodes draining the primary tumor and risking metastatic invasion. They are representative of axillary lymph node basin status, providing information on the indication of further node clearance and the requirement of adjuvant therapies. If SLNs are not involved, axillary lymph node dissection can be avoided.

The SLN clinical procedure usually consists in the injection of a lymphophilic radiolabeled tracer and of a blue dye. After skin incision in the armpit, SLNs are then localized with a nuclear probe and by visual inspection prior to biopsy and histological analysis [3]. In breast cancer, periareolar injection of the radiotracer and dye results in a very high SLN detection rate (99.1%) [4].

Further improvement of SLN detection methods should still be considered for two main reasons: to increase the specificity in detecting nodes invaded by metastasis and to simplify the surgical procedure. Withdrawing radioisotopes can be considered by using alternative: magnetic, ultrasonic or optical methods for tracer detection. The development of new optical methods is a fast growing research field. Such methods can be based on the measurement of backscattered light, on the detection of fluorescence photons [5–11] or measurement of the acoustic waves generated by the photoacoustic effect [12,13], or a combination of these [9,14]. Several tracers can be used, such as dyes, fluorescent molecules, quantum dots [15,16] or nano-objects for multimodal imaging [17–19]. They should have one absorption band in the red or near infrared range where light has its maximum depth of penetration in tissues.

In order to speed up the clinical application of a new optical method, we considered the use of a tracer already approved by regulation authorities. This restricts the choice to some blue dyes or to Indocyanine Green (ICG), a fluorescent probe used in medical diagnosis to evaluate the cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography. ICG can only be delivered through intravenous injection and requires preliminary authorization before clinical studies in SLNs detection. Moreover, many studies use ICG fluorescence combined with blue dye coloration for comparison purpose [9,11].

We decided to use the optical detection of blue dyes already in use for SLNs detection by visual inspection, such as Isosulfan Blue in the USA or Patent Blue V (PBV) in Europe. It is commonly recognized that these dyes are not fluorescent and we therefore developed a method based on the detection of backscattered photons [20,21]. The biodistribution and pharmacokinetics of these dyes strongly depend on their binding to lymphatic proteins such as albumin. Patent Blue V is a triaryl methane dye that weakly binds to albumin [22], probably through its two sulphonic acid groups [23]. It is also known that the binding sites of albumin are very efficient in preventing fast non-radiative relaxation processes via rotational motion of the aromatic rings of the triarylmethanes, resulting in an enhanced fluorescence quantum yield [24].
In this paper, we will highlight the fact that it is possible to amplify the fluorescence quantum yield of PBV, by binding it with Human Serum Albumin (HSA), prior to percutaneous injection of the mixture. We will present the photophysical properties of free and HSA-bound PBV. The fluorescence detection of the PBV/HSA mixture was evaluated in scattering test objects and in an animal model with inflamed nodes, using a mono-pixel photodetector and a CCD camera.

2. Materials and methods

2.1. Imaging agents

Patent Blue V sodium Guerbet 2.5 per cent solution was used. The solution is packaged in 2-mL glass ampoules and is ready for subcutaneous injection. It corresponds to PBV sodium salt having a molecular weight of 582.7 Da. Spectroscopic experiments on free PBV were performed after dilution in phosphate buffered saline (PBS).

HSA solutions were prepared from Sigma Aldrich HSA lyophilized powder dissolved in PBS. The molecular weight is 66478 Da. The HSA concentration in adult serum lies between 38 and 48 g × L\(^{-1}\) and the lymph/plasma concentration ratio is close to 0.5 [25]. Preclinical studies were either performed with Sigma HSA solutions or with the commercial form Vialebex (LFB Biomedicaments), a 200 g × L\(^{-1}\) HSA solution available for injection in humans.

PBV and HSA were extemporaneously mixed before spectroscopic analysis or subcutaneous injections. The fraction of dye bound to HSA was measured by delivering the solution into one side of a dialysis cell partitioned with a cellulose membrane Spectra/Por (Spectrum) of 3,500 Da molecular weight cut off. The dialysis samples were incubated for 12 hours. Absorbance at 638 nm of the dialyzed solution was recorded to compute the free and HSA-bound dye concentrations.

PBV was also mixed with Nanocoll (GE Healthcare), a nanocolloidal HSA suspension used for SLN node detection after labeling with technetium 99m. No fluorescence was observed on this mixture and dialysis equilibrium studies demonstrate that PBV does not bind on this denatured albumin.

Preliminary studies on scattering phantoms were carried out on solutions prepared from Intralipid (Fresenius Kabi) 20% emulsion colored with Black India ink (Lefranc & Bourgeois). Intralipid and ink concentrations were adjusted to obtain the required absorption and scattering properties [26]. To simulate breast tissues, we chose a reduced scattering coefficient (\(\mu_s^0\)) equal to 0.9 mm\(^{-1}\) and an absorption coefficient (\(\mu_a\)) equal to 0.005 mm\(^{-1}\) at 640 nm [27]. PBV, free or bound to albumin, was finally added by successive injections.

2.2. Spectroscopy

A Uvikon 943 (Kontron) double-beam UV-Vis spectrophotometer was used for all absorption spectroscopy measurements and solution preparation. Fluorescence excitation and emission spectra and fluorescence quantum yield (\(\phi\)) measurements were performed on a FluoroMax-3 spectrofluorometer (Jobin-Yvon Horiba) at room temperature. All spectra were corrected for intensity variations of the excitation source.

The fluorescence quantum yield \(\phi\) was determined by a comparison method, requiring prior knowledge of the fluorescence quantum yield of a reference dye [28]. The chosen reference dye, Nile Blue (Sigma Aldrich), exhibits both absorption and emission spectra close to PBV spectra, with absorption and emission maxima at 626 and 660 nm, respectively and a quantum yield of 0.27 [29].

2.3. In situ fluorescence detection

A fluorescence detection system was assembled in the laboratory (Fig. 1). The light source was a laser diode (RLTMRL-III-635, Roithner Lasertechnik GmbH) emitting at 635 nm with
an output power adjustable between 1 and 500 mW. The laser diode was coupled to an optical fiber via an SMA connector. A bandpass interference filter (Edmund Techspec) centered at 632 nm, with a 10-nm full width at half maximum (FWHM) and an optical density (OD) greater than 3 in the rejected band, eliminated emitted photons whose wavelength is greater than 637 nm.

The probe for performing in situ fluorescence measurements was made of stainless steel by Eurorad SA. It included two optical fibers for emission and reception, separated by a 4-mm gap.

A photosensor module, containing a photomultiplier tube (PMT) and its high-voltage power supply (Hamamatsu H10721-20), was used for detecting fluorescence photons. A longpass filter (Edmund Techspec High Performance) at 650 nm, with an OD greater than 4 in the rejected band, was placed in front of the PMT photocathode to eliminate scattered excitation photons.

The laser diode was controlled via a multifunction data acquisition (DAQ) device (National Instrument NI-USB-6229) connected to a laptop, using the Labview software (National Instrument). The laser beam was modulated at a frequency, typically 820 Hz. Signal from the PMT was digitalized by the same DAQ device. The fluorescence signal was filtered out of the noise and any other optical signals, non-modulated at 820 Hz, by a frequency analysis, Fast Fourier Transform (FFT) or Lock in Detection. We used FFT analysis in the experiments described in this paper.

![Fig. 1. Schema of the single probe setup.](image)

2.4. Fluorescence imaging

The fluorescence imaging device used in this study was initially developed by Eurorad SA, for preclinical imaging and has been adapted for the experiments with PBV (Fig. 2). It contains a ring of white light-emitting diodes (LED) for lightening the region of interest. The laser diode at 635 nm and the bandpass filter described previously were used for PBV fluorescence excitation. The optical fiber was connected to a frontal light distributor (Medlight SA) to obtain a uniform circular illumination spot. Experiments were conducted with an excitation power of about 200 mW distributed over an illuminated 12.5 cm² area. A camera lens, followed by a beam-splitter prism, was used to form the images of the object on two sensors. The first one was the CCD sensor of a camera (Pixelfly QE) that acquired the color image of the object. The second sensor, placed behind a highpass filter at 700 nm, was the CCD sensor of a camera (AVT Stingray) used for acquiring the fluorescence image. All images presented below were acquired with an 80-ms exposure time. The two images were transferred to a computer for real-time treatment, superimposition and storage, using a graphical interface developed with the Labview software (National Instrument). The object, or animal, and the device were placed within a black box. During preclinical experiments, the animal was laid on a temperature-controlled bed and anesthetized with a mixture of 2% isoflurane and oxygen, with the use of an anesthesia system and a temperature control unit (Equipement Veterinaire Minerve).
2.5. Animal preparation

The animal experiment protocol was approved under N° AL/01/17/10/09 and conformed to the French regulation n° 87.848 of October 19th, 1987.

Lewis rats (Janvier), 6 to 8 weeks old, were studied. Inflamed lymph nodes were produced by immunization of the animals. Rats were anesthetized with an intraperitoneal injection containing 100 mg.kg\(^{-1}\) of ketamine and 10 mg.kg\(^{-1}\) of xylazine. Rats were inoculated subcutaneously in both hind limbs with an emulsion of myelin basic protein and complete Freund’s adjuvant (MBP/CFA). CFA was prepared by adding 4 mg of Mycobacterium tuberculosis strain H37RA (Difco) per milliliter of incomplete Freund’s adjuvant (Sigma F5506). The antigen solution was prepared from MBP at 2 mg.mL\(^{-1}\) in PBS. This model is known to induce acute experimental allergic encephalomyelitis (EAE) [30].

Fluorescence detection of the lymph nodes was performed ten days after immunization, a delay corresponding to the maximum severity of the clinical signs, i.e, paraplegia with incontinence, before their progressive diminution. The PBV/HSA solution was injected subcutaneously in the left hind limb. The PBV/HSA mixture was drained preferentially to the popliteal, inguinal and iliac nodes [31].

3. Results and discussion

3.1. Photophysical properties of PBV

Spectroscopic measurements were performed on PBV solutions with a 14 µmol × L\(^{-1}\) dye concentration. When mixed with HSA solutions at concentrations greater than 0.5 mmol × L\(^{-1}\) (33 g × L\(^{-1}\)), all the dye was bound, as verified by equilibrium dialysis. The spectra of free and HSA-bound PBV (40 g × L\(^{-1}\)) are presented in Fig. 3. The absorption spectrum of free PBV in PBS (pH = 7.4) exhibited a maximum, in the red part of the spectrum, at 638 nm, with a molar extinction coefficient close to 120,000 mol\(^{-1}\) × L × cm\(^{-1}\). The binding of the dye to HSA induced a slight bathochromic shift (2 nm) of the maximum absorption and a slight hypochromic effect on its maximum molar extinction coefficient (100,000 mol\(^{-1}\) × L × cm\(^{-1}\)).

PBV is widely used for its coloring properties but is known as a non-fluorescent dye. Its fluorescence quantum yield $\phi$ was reported to be 3.9 × 10\(^{-4}\) [32,33]. Triarylmethane molecules, such as PBV, have an extremely low $\phi$ due to their fast vibrational de-excitation process. However, fluorescence can be enhanced by restricting vibrational modes, for
example by dissolving molecules in a viscous environment or by binding them on macromolecules like proteins [24]. Other relaxation pathways could also be amplified, such as the increased intersystem crossing to the triplet state, which will induce photochemical reactions leading to singlet oxygen and free radical production [34]. Figure 3 shows the emission spectra of free PBV (4 µmol × L\(^{-1}\)), and of the dye (4 µmol × L\(^{-1}\)) bound to HSA (33 g × L\(^{-1}\)). The maxima of the emission spectra are both situated at 660 nm, but the amplitude of the fluorescence signal of the PBV/HSA complex was largely increased as compared to the corresponding signal of the free dye.

![Absorption and emission spectra](image)

Fig. 3. Absorption spectra of: (a) free PBV (black curve) and (b) HSA-bound PBV (blue curve). Emission spectra of: (c) PBV, 4 µmol × L\(^{-1}\), bound to HSA 0.125 mmol × L\(^{-1}\) (red curve) and (d) free PBV (green curve) with a 620-nm excitation wavelength.

The PBV quantum yield \(\phi\) was measured at 2 excitation wavelengths. The first one was 585 nm, the shoulder of the absorption spectrum. The second one, 620 nm, was selected as the wavelength where optical densities of the PBV and Nile Blue solutions were equal. Using the comparison procedure, the quantum yield \(\phi\) measured for free PBV was 5.3 × 10\(^{-4}\) and 4.7 × 10\(^{-4}\) at 585 nm and 620 nm excitation wavelengths, respectively. It is slightly higher than the published value. The quantum yield of PBV bound to HSA was measured with protein concentrations varying from 6.25 × 10\(^{-2}\) mmol × L\(^{-1}\) to 1 mmol × L\(^{-1}\). We observed a constant quantum yield, corresponding to fully bound dye, at all HSA concentrations. The mean \(\phi\) value was equal to 1.7 × 10\(^{-2}\) ± 0.3 × 10\(^{-2}\) at 585 nm, and to 1.2 × 10\(^{-2}\) ± 0.2 × 10\(^{-2}\) at 620 nm. The corresponding amplification coefficient, defined as the ratio between the quantum yields \(\phi\) of HSA-bound and free PBV, was 32 and 26 at 585 and 620 nm respectively.

The low quantum yield of the free dye precludes any in-vivo detection of PBV fluorescence. Mixing the dye with a protein like HSA results in a strong amplification of fluorescence that allows considering new in vivo applications. The fluorescence quantum yield of HSA-bound PBV is much lower than that of other fluorescent dyes used in preclinical imaging, such as cyanine dyes Cy5.5 and Cy7 having quantum yields of 0.24 and 0.28, respectively [35]. But this quantum yield is close to that of ICG, one of the most used dye for clinical fluorescence. The quantum yield of free ICG, in PBS solution is about 2.7 × 10\(^{-2}\) [36], while it increases to 9.3 × 10\(^{-2}\) once bound to HSA [5].

Those interesting photophysical properties prompted us to evaluate fluorescence detection and mapping of SLNs with PBV/HSA solutions.

### 3.2. PBV/HSA fluorescence detection

The fluorescence detection system previously described (Fig. 1) was assembled with the aim to provide surgeons with a simple diagnostic tool, able to localize the SLN without changing significantly their practice. Preliminary experiments were devoted to the determination of sensitivity as regards the detection of PBV fluorescence signals. In order to compare the results with the sensitivity of PBV detection with backscattered photons measurements, these
experiments were performed on scattering Intralipid solutions. Figure 4(a) shows the intensity of the fluorescence signal measured as a function of the PBV concentration in the Intralipid solution, with a 20-mW excitation light power. Each point corresponds to the mean of data acquired over a one minute measurement, for a given dye concentration. The error bars correspond to ± 2 standard deviations.

We can notice signal when the probe is facing a nonfluorescent solution, which is due to incomplete filtering of backscattered excitation photons. Better optical filtering will reduce this “nil” signal and improve the detection sensitivity. The detection threshold was determined with a receiver operating characteristic (ROC) analysis [37] as presented in Fig. 4(b). One group of data corresponds to all the acquisitions performed for a given dye concentration. Solution without PBV/HSA was used as the reference group (GREF), other groups (G1, G2,..., Gi,...) were obtained after injection of the dye, i being the number of injections. G4 (25 pmol × L⁻¹) corresponds to a sensitivity (Se) and a specificity (Sp) greater than 80%. G5 (31 pmol × L⁻¹) allows to reach Se and Sp greater than 95%. Whatever the choice, G4 or G5, the sensitivity increases by a factor 10³ compared to the eye visibility threshold estimated at about 300 nmol × L⁻¹.

Fig. 4. (a) Variations in the fluorescence signal measured as a function of PBV concentration (laser power 20 mW), (b) ROC curves for the determination of the detection threshold.

The power limit of class 3R lasers, usable in clinics hardly without necessity to implement operating precautions, is set at 5 mW for continuous lasers in the visible range. We therefore repeated the previous study with that output power. The measured detection threshold was 2.5 nmol × L⁻¹. This result is not in agreement with the linear relation between fluorescence and excitation intensities. This is probably due to imperfect filtering of backscattered photons generating background signal.

The sensitivity of PBV detection in Intralipid solutions, by fluorescence, can be compared with the detection threshold using scattered photons [20,21]. With that method, we obtained a detection threshold equal to 10 nmol × L⁻¹, with a 1-mW illumination power. The results are very close and we can consider that the fluorescent complex PBV/HSA can be detected with a sensitivity equivalent, or even better, providing optical filtering is improved, to that obtained with backscattered photon detection, a method already successfully evaluated during ex-vivo experiments on excised SLN [38].

The method using PBV/HSA fluorescence detected with a fiber optical probe-based device presents the double advantage of working with a dye already used in clinical routine and with an optical device that can be used in the same way as a nuclear probe. Dual-modality SLN detection, by fluorescence and visual inspection, can thus be performed with a single tracer. The optical fibers could also easily be integrated in a nuclear probe, providing surgeons with a tri-modal SLN detection procedure.
3.3. PBV/HSA fluorescence imaging

The following results were obtained with the imaging system described in the previous section and illustrated on Fig. 2. The current objective is to provide surgeons with a device to localize, in the operative field, a node labeled with the fluorescent complex PBV/HSA.

First, experiments were performed on test objects to evaluate the sensitivity of PBV detection with fluorescence imaging. Experiments using either HSA from Sigma or the pharmaceutical preparation Vialebex yielded identical results. Solutions were prepared in 250-µL micro-vials, with 65 g × L⁻¹ HSA Sigma or Vialebex. Figure 5(a) presents the color image of 7 micro-vials, containing different PBV/Vialebex mixtures. The first vial (a) contains only Vialebex. The second one (b) contains free PBV, at 5 µmol × L⁻¹. The next vials contain Vialebex with the following PBV concentrations: 1 µmol × L⁻¹ (c), 2.5 µmol × L⁻¹ (d), 5 µmol × L⁻¹ (e), 7.5 µmol × L⁻¹ (f), 10 µmol × L⁻¹ (g). Figure 5(b) shows the fluorescence image obtained upon illumination with the laser diode at 635 nm, with incident intensity equal to 16 mW.cm⁻². The red circles represent the regions of interest where the fluorescence signal was measured for quantitative analysis. Figure 5(c) presents the fluorescence image superimposed on the color image.

Increasing fluorescence intensity can be observed, from the micro-vial (c) to the micro-vial (g), with a progressive signal saturation. The fluorescence intensity profile measured in the regions of interest, Fig. 5(d), presents the same information. The saturation observed was followed by a decrease in fluorescence signal at higher PBV concentrations (data not shown). This result may be mainly due to the self-absorption of fluorescence photons by the dye (screening effect), a 10-µmol × L⁻¹ PBV concentration corresponding to an optical density close to 1 at 635 nm. Imaging experiments were also performed on micro-vials containing mixtures of HSA-bound PBV at 5-µmol × L⁻¹ concentration immersed in Intralipid solutions. The image of the vials, enlarged by the scattering of fluorescence photons was easily observed, through Intralipid layers up to 2-cm thick.
An animal experimentation was carried-out on 9 Lewis rats, sensitized according to the procedure described in the Materials and Methods section. Swollen inflamed nodes, sizing about 5 mm, were palpable on the day of the imaging experiment.

Figure 6(a) represents the color image and Fig. 6(b) the fluorescence image recorded after partial depilation of a rat injected with 100 µL of a Vialebex (3 mmol × L$^{-1}$) – PBV (100 µmol × L$^{-1}$) mixture. The subcutaneous injection was made in the hind limb of anesthetized animals, about 5 minutes before imaging. The superimposition of both images can be observed on Fig. 6(c). The inguinal lymph node can be clearly localized, under the skin of the animal. Detection of this inflamed node was effective for all studied rats, with or without prior shaving.

Fig. 6. Images of rats injected with PBV/Vialebex (top) and PBV/rat serum albumin (bottom). Color images (a, d), fluorescence images (b, e) and overlaid images (c, f) showing the inguinal lymph node.

Two other albumins were used to record fluorescence images of inflamed nodes on rats: Sigma Aldrich HSA and rat serum albumin. In all cases, we could observe a fluorescence image. Figure 6(d) represents the color image, Fig. 6(e) the fluorescence image, recorded without depilation of the rat, and Fig. 6(f) the superimposed images. That animal was injected with a 20-µmol × L$^{-1}$ PBV solution, in rat serum. The inguinal lymph node can be readily localized in this case. The popliteal lymph node was observed on animals injected with higher PBV concentrations.

As control group two animals were injected with free PBV and followed with fluorescence imaging during a 20 minutes period. In both cases, no fluorescence signal was observed, suggesting that the dye does not bind with serum albumin in-vivo and that the pre-mixture is required. This observation can be attributed to the fact that serum albumin already binds many classes of ligands. Its exogenous/endogenous ligand binding preference is a very active field of research in therapeutics and clinical biochemistry.

4. Conclusions

PBV is a clinically available dye for SLN detection. When mixed with HSA prior to use, the fluorescence quantum yield of the dye was increased enough to allow its detection and imaging by near infrared fluorescence techniques. This work demonstrates that fluorescence photons emitted by HSA-bound PBV can be detected in vivo, and that inflamed nodes can be localized, using an animal model.

The excitation wavelength, 635 nm, used for in vivo investigations is high enough to allow a good penetration of incident light in tissues. The fluorescence quantum yield of the dye, when bound to HSA, is about 1.7 × 10$^{-2}$ with maximum emission at 660 nm. This
quantum yield is about two times lower than that of free ICG, the most widely used fluorescent dye for SLN mapping. This lower fluorescence yield and the lower excitation and emission wavelengths, compared to ICG values, mean that the depth at which the dye can be detected in tissues is reduced. But these drawbacks can be minimized when SLN detection is performed during surgery.

The main advantages of the proposed method are the use of a dye being approved for clinical use in Europe, and the possibility of SLN co-registration with a fluorescence optical device and by visual inspection during surgery.

The lower contribution of non-radiative relaxation pathways of excited PBV once bound to HSA is responsible for the higher fluorescence of the complex. But the increased intersystem crossing to the triplet state could induce phototoxicity which should be studied, both for photoprotection that would be required after surgery, or for a possible photodynamic therapy using HSA-bound dyes.

Several experiments were performed to measure the photophysical properties of the dye, free and bound to HSA. A detection setup based on an optical fiber probe was assembled. It is similar in shape to nuclear probes used in clinics, and can be easily implemented in a nuclear probe to allow multi-modality SLN detection. Using this device on scattering phantoms, mimicking tissues, we were able to detect PBV with concentrations as low as 25 pmol × L\(^{-1}\), with a 20-mW light power. The detection sensitivity is much lower than the eye visibility threshold, estimated at 300 nmol × L\(^{-1}\) on the same object.

This work was completed by fluorescence imaging experiments using a preclinical prototype, adapted for the detection of PBV fluorescence. Experiments on test objects demonstrated that the “ideal” PBV concentration range, for optimal detection sensitivity, was between 1 and 10 µmol × L\(^{-1}\). Preclinical experiments with PBV mixed with various HSA, including Vialebex, a pharmaceutical preparation for human clinical use, demonstrated that inflamed nodes could be localized, even through animal fur.

The proposed SLN detection and mapping technique could be an alternative to the traditional method, which involves isotopic detection, with all inherent protection, regulation and costs issues. It also allows to combine a fluorescence detection technique and visual inspection of blue nodes, using only one tracer, thereby avoiding increased potential hazard due to tracer cocktail injections. It should be noticed that large epidemiologic researches have been conducted on potential adverse effects after PBV injections. Out of 7,917 patients, only 0.9% experienced adverse reactions. Severe reactions were noted in 5 (0.06%) patients but without mortality [39]. HSA should be used with caution as it is made from human plasma and may contain infectious agents, such as viruses. The risk to transmit an infectious agent with HSA preparation approved for clinical use is almost fully eliminated by screening plasma donors, by testing for the presence of virus infections, and by inactivating viruses by pasteurization. Despite these measures, HSA can still potentially transmit disease, and the physician should discuss the risks and benefits of this product with the patient prior to injection. We therefore expect to be in a position in the near future to initiate clinical research, either by using a dual, nuclear and fluorescence detection probe, or with a fluorescence imaging clinical device adapted to PBV detection.

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