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So much promise, so little delivery: Underuse of biological evidence collected from sexual assault victims treated in an Italian specialised hospital centre

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ABSTRACT

In an Italian hospital centre treating victims of sexual assault, requests for genetic testing of biological samples collected during forensic medical examinations (GT cases) were identified from 2003 to 2023, and details of the results of body fluid identification (BFI) and DNA analysis were obtained from the designated laboratories. Elements that could influence the decision to perform forensic DNA analysis were derived from the medical records of GT cases and a comparison group of patients whose samples were not genetically tested.

The overall proportion of GT cases was 7.8 %, remaining limited even after the implementation of the National DNA Database (BDN-DNA) in 2017 (14.3 % in 2023). Items that were significantly more common in GT cases compared to the comparison group included: a positive sperm microscopy result; short time since intercourse (TSI) (<24 h in >80 % of GT cases); reported ejaculation; aggression by a stranger; group violence; absence of injuries.

Male DNA profiles were obtained from 52.1 % of samples using a direct-to-DNA approach, and from 39.2 % using a traditional strategy involving differential extraction, depending on the preliminary BFI results. The success rate was significantly higher for shorter TSI and positive BFI test for semen, although male DNA profiles could be obtained in 18–27 % of semen negative samples. Only 25.0 % of eligible DNA profiles obtained after 2017 were uploaded to BDN-DNA.

The results show the underuse of biological evidence in the investigation of sexual violence cases in the Italian criminal justice system and the lack of awareness of the potential of BDN-DNA.

1. Introduction

Sexual violence is often associated with the deposition of biological traces of the perpetrator on the victim's body [1]. Forensic medical examination (FME) allows these traces to be collected and preserved for subsequent laboratory testing, including body fluid identification (BFI) and DNA analysis to characterise the genetic profile of the perpetrator [2,3]. BFI can be performed using highly sensitive but nonspecific presumptive methods, such as alternative light sources and chemical/catalytic tests, followed by highly specific confirmatory tests, including microscopy to confirm the presence of sperm, immunological and biomolecular assays [3,4]. The genetic analysis workflow begins with DNA extraction, which can be either standard or 'differential', separating the DNA components into a sperm (male-specific) fraction

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and an epithelial fraction (containing DNA from both male and female cells) [5]. This is usually followed by DNA quantification, initially by probe hybridisation and nowadays based on real-time PCR (RT-PCR), which can allow separate determination of total and male DNA concentrations, detection of PCR inhibition and assessment of the degree of DNA degradation in the sample [6]. Finally, PCR amplification of short tandem repeat (STR) loci, either autosomal (aSTR) or Y-chromosomal (Y-STR), depending on the ratio of male to female DNA in the DNA extract, is performed to obtain genetic profiles for comparison with one or more suspects.

In Italy, the FME for sexual assault is generally carried out in the emergency departments of general hospitals [7,8], which may or may not have dedicated paediatric [9] and obstetric-gynaecological [10] emergency services. Very few hospitals have a specialised centre dedicated exclusively to the care of victims of sexual violence, with a 24-hour on-call service [11,12]. In Italy, national guidelines aimed at standardising the care pathway in emergency departments for victims of violence have been available since 2017 [13]. They include guidance on the collection of biological evidence in accordance with the recommendations of the Italian Working Group of the International Society for Forensic Genetics (GEFI) [14], recently updated [15].

The fate of the biological samples collected during the FME then depends on several factors. According to the Italian Criminal Code, sexual violence is a crime against personal liberty and therefore a type of crime that is prosecuted on complaint. This means that it is up to the victim to ask the judicial authority (public prosecutor) - directly or through the police - to open an investigation. However, there are many exceptions to this principle. For example, if the victim is a minor, if the violence is accompanied by serious injuries, in the case of group violence or attacks facilitated by the use of drugs or alcohol, the health professionals who carried out the FME are obliged to inform the public prosecutor, who must open an investigation ex officio [10].

Police forces involved in sexual assault investigations can request permission directly from the public prosecutor to carry out genetic analyses of samples collected during FME in their own laboratories. Alternatively, the public prosecutor may instruct experts at universities or private laboratories to conduct the genetic investigations. Since January 2017, a National DNA Database (Banca Dati Nazionale del DNA, BDN-DNA) has been operational in Italy to compare aSTR profiles and Y-STR haplotypes obtained from crime scenes, including those related to events of sexual violence, and from persons arrested or detained for the commission of particularly serious crimes, including sexual violence [16]. Law No. 85 of 2009 establishing the BDN-DNA provides for annual reports on its activities. However, the data currently available is still scarce and not very detailed, with around 50 perpetrators of sexual violence apparently identified through the BDN-DNA as of February 2024 [17]. There is also a lack of multi-centre studies [18] or large single-centre studies [11,12,19] carried out in Italian hospitals providing services to victims of sexual violence, reporting the proportion and characteristics of cases subjected to genetic testing and examining the outcome of DNA investigations. These studies, which were conducted before the implementation of BDN-DNA, show an underuse of forensic genetic investigation tools, with more than 90 % of biological samples collected during FME being stored in hospital facilities without further analysis [11,12].

This study retrospectively analysed the case history from 2003 to 2023 of the Sexual Violence Support (SVS) centre of the University Obstetric-Gynaecological Hospital of Turin (UOGHT), which takes care of female victims of sexual violence over the age of 14 in the Piedmont region (north-west Italy). The medical records of patients whose biological samples collected during the FME were subjected to genetic testing were compared with those of cases in which genetic testing was not requested, in order to identify possible factors influencing the judicial authority's decision. The methods used and the results obtained by the laboratories responsible for genetic analysis were also reviewed. The ultimate aim is to provide the Italian National Health Service and the judiciary with useful elements to rationalise the management of biological sampling at the time of FME of sexual assault victims and to maximise the effectiveness of genetic investigations, also in the light of the new potential offered by BDN-DNA.

2. Materials and methods

2.1. Clinical data from SVS cases with and without genetic testing

The medical records of patients who received support at the SVS centre following a reported sexual assault in the period 2003–2023 and who underwent collection of biological evidence during the FME were examined (n = 1175). The time between the collection of biological samples and the reported sexual violence (time since sexual intercourse, TSI) and the results of microscopic examination to detect the presence of spermatozoa, routinely performed at the UOGHT Cytology Service (CS-UOGHT) on intimate samples from SVS patients, were recorded. The subset of cases that underwent forensic genetic testing (n = 92) was then identified by reviewing the SVS chain of custody archive. The medical records of this subset of cases, henceforth referred to as genetically tested (GT) patients/cases, were further examined to obtain the following data: type of aggression (with or without reported ejaculation or use of condom); type of aggressor (single/multiple, stranger/non-stranger to the victim); whether the compliant mentioned bathing or showering after the aggression; presence/absence of amnesia; presence/absence of physical injuries; type of evidence collected; report to the judicial authority by SVS health personnel. The same data were collected from a comparison group of SVS patients (not genetically tested, NGT patients/cases) who underwent collection of biological evidence during FME, but whose samples were not subjected to genetic testing due to the lack of a request from the police and/or judicial authority. Specifically, 20 % of the cases for each year studied, evenly distributed over the year, were randomly selected to form this reference sample (n = 218).

2.2. Laboratory analyses of GT cases

The SVS chain of custody archive was used to identify police laboratories and/or individual experts from police, university and private laboratories who had been appointed by the judicial authority to carry out genetic testing on collected evidence. The laboratory/expert was contacted and provided with the unique SVS code assigned to each case, which identified the evidence when it was transferred from the hospital to the laboratory/expert for genetic testing. For each case, the laboratories/experts were asked to return the following information in an anonymous form to avoid any link to a specific SVS patient: period of analysis (before or after activation of BDN-DNA); type of request for genetic testing (initiated by the police laboratory and approved by the prosecutor or expert opinion); type and results of BFI tests performed; DNA isolation techniques; DNA quantification techniques and results; targeted DNA markers (aSTR and/or Y-STRs); DNA typing results (single/mixed aSTR profiles and/or Y-STR haplotypes, completeness of DNA profiles); availability of reference samples from one or more suspects; results of DNA comparisons (exclusion/inclusion as source/contributor to single donor or mixed aSTR/Y-STR profiles); relevant results of genetic testing of any case-related evidence not derived from FME of victims carried out by SVS (e.g. crime scene samples); inclusion of DNA profiles in the BDN-DNA.

2.3. Statistical analysis

Statistical processing of the data (Chi-square test to compare frequencies, T-test and Mann-Whitney *U* test, depending on normality, to compare distributions) was performed using Jamovi 2.3.21 software.

2.4. Ethical approval

The retrospective analysis of SVS medical records was approved by the Ethics Committee of UOGHT (n° 581/2023).

3. Results

3.1. SVS cases

Of 1175 patients examined at the SVS centre in the period 2003–2023 who underwent collection of biological evidence during FME, only 92 (7.8 %) had their samples subjected to genetic testing following authorisation/request by the judicial authority. The absolute number per year of FMEs with collection of biological evidence and GT cases is shown in Fig. 1, together with the annual and total proportions of GT cases. The average annual number of sexual assault victims who underwent a FME with collection of biological evidence was 56.0 ± 10.8 SD, and that of GT cases was 4.4 ± 2.6 SD. The percentage of GT cases reached a maximum of 21.1 % in 2005, followed by a steady decline to 0 % in 2017, when samples from none of the 61 sexual violence victims receiving assistance were further analysed. The percentage of GT cases remained constant thereafter (4–5 % per year), with a new increase in 2023 (14.3 %).

3.2. Characteristics of GT and NGT cases derived from SVS medical records

The box plots in Fig. 2 show the distribution of TSI measured in hours (log scale) in GT cases compared to NGT cases for which information was retrieved from medical records (n = 1060). Median and

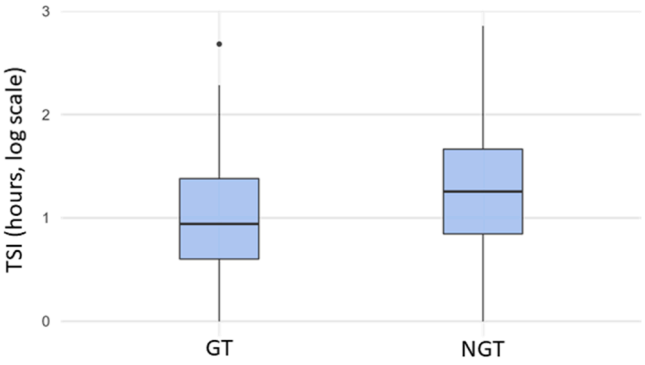

Fig. 2. Box plot of TSI distribution (hours, log scale) in GT and NGT cases.

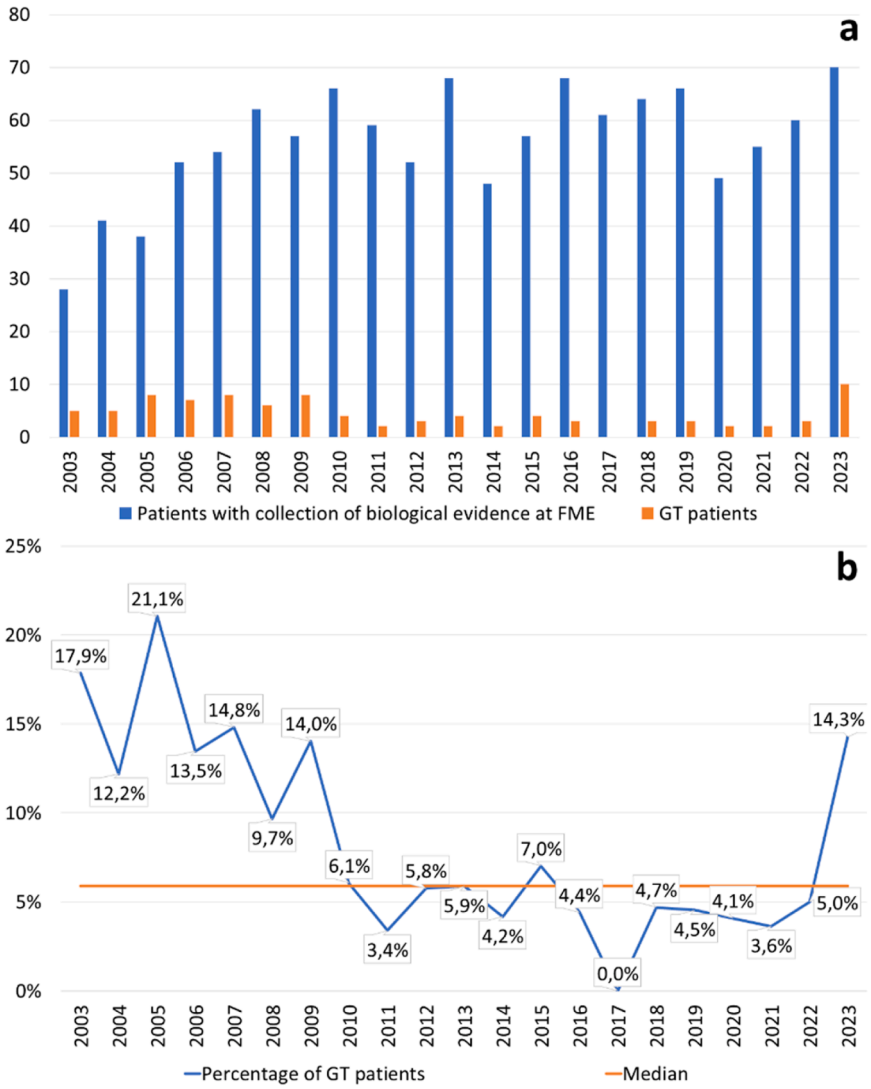

Fig. 1. The absolute number per year of patients undergoing collection of biological evidence during FME at the SVS centre and the number of GT cases are shown in a); the number per year and the median (2003–2023) of GT cases are shown in b).

interquartile range (IQR) were 8.75 h (IQR 4–24) for GT cases and 18 h (IQR 7–46.25) for NGT cases. TSI was significantly shorter in GT compared to NGT cases (T-test, $p < 0.001$). In particular, TSI > 24 and > 48 h were observed in only 18.5 % and 12 % of GT cases, respectively. On the other hand, TSI > 24 and > 48 h were reported in 37.5 % and 21.3 % of NGT cases, respectively.

The presence of spermatozoa was preliminarily assessed at CS-UOGHT by microscopic examination in 79 GT cases and 1054 NGT cases. The proportion of positive results was significantly higher in GT (34.2 %) compared to NGT cases (15.0 %) (Chi-square test, $p < 0.001$).

Comparisons of other elements derived from the medical records of SVS patients in GT and NGT cases are summarised in Table 1.

Conditions that were significantly more common in GT cases than in NGT cases were: reported ejaculation, perpetrator being a stranger to the victim, group violence, and the presence of conditions leading to a duty to report to the judicial authority by health professionals. The opposite was found for the association with physical injury.

3.3. Genetic analysis of GT cases

3.3.1. General characteristics of GT cases

Data on 74 out of 92 GT cases (80.4 %) were obtained from the designated laboratory/expert. DNA testing was carried out in four different facilities, including university laboratories (49 cases), police laboratories (17 cases) and a private laboratory (8 cases). In most cases, DNA testing was carried out in the form of an expert opinion requested by a prosecutor (75.7 %) or a judge (8.1 %). Only 16.2 % of the investigations were initiated by police laboratories authorised by the public prosecutor.

A total of 366 pieces of evidence were analysed (average 5.0 ± 2.5 SD per case, with the number of samples ranging from 1 to a maximum of 10). The most common types of evidence were intimate swabs from the cervicovaginal cavity (36.6 %), external genitalia including the perineum (12.0 %), oral cavity (4.9 %) and the ano-rectal cavity (4.6 %). Other biological samples included skin swabs (3.3 %), subungual scrapings (2.5 %) and hair (0.3 %). The remaining evidence was clothing (18.9 %) and other material (16.9 %). The latter consisted mainly of stained and unstained microscopic slides obtained from intimate swabs (58.1 %), followed by condoms (9.7 %) and sanitary towels (8.1 %).

Table 1
Proportion of relevant features derived from medical records of GT and NGT cases with p-value of Chi-square test comparisons. For each feature, the number of cases for which information could be obtained from the medical records is given in the 'n' columns.

	n	GT	n	NGT	*p*
Type of aggression - ejaculation	36		83		**< 0.05**
Yes		80.6 %		59.0 %	
No		19.4 %		41.0 %	
Type of aggression - condom	53		135		> 0.05
Yes		5.7 %		8.9 %	
No		94.3 %		91.1 %	
Type of aggressor	90*		208		**< 0.05**
Stranger		61.1 %		42.3 %	
Non-stranger		38.9 %		57.7 %	
Group violence	91		194		**< 0.05**
Yes		31.9 %		19.1 %	
No		68.1 %		80.9 %	
Amnesia	90		217		> 0.05
Yes		34.4 %		30.4 %	
No		65.6 %		69.6 %	
Physical injuries	92		217		**< 0.05**
Yes		53.3 %		65.4 %	
No		46.7 %		34.6 %	
Bathing or showering	92		201		> 0.05
Yes		46.7 %		56.7 %	
No		53.3 %		43.3 %	
Report to judicial authority	92		218		**< 0.05**
Yes		94.6 %		83.0 %	
No		5.4 %		17.0 %	

*In one case of group violence, the perpetrators included both strangers and non-strangers.

3.3.2. BFI

A total of 448 samples were taken from the evidence examined, as some items of clothing and other objects were sampled more than once. BFI was carried out on 77.2 % of the samples. The vast majority consisted of immunochromatographic tests for semen and/or saliva (84.1 %), followed by microscopic examination to detect spermatozoa (11.8 %), use of alternative light sources (3.5 %) and chemical-enzymatic presumptive tests for semen (0.6 %). No laboratory/expert attempted BFI by biomolecular testing targeting mRNA, miRNA or tissue-specific epigenetic/microbial signatures.

For 33 intimate swabs, the microscopic examination to assess the presence of spermatozoa performed at CS-UOGHT was repeated by the designated laboratory/expert, either on duplicate swabs or on corresponding stained/unstained slides provided with the evidence. Results were concordant in 90.9 % of cases. Of the three discordant results, two were from a single case where the designated laboratory/expert did not observe spermatozoa on slides obtained from cervicovaginal swabs that had been found positive by CS-UOGHT. The opposite happened with an ano-rectal swab from another case. Of 90 intimate swab specimens in which the microscopic examination performed by CS-UOGHT was re-evaluated by means of immunochromatographic testing on duplicate swabs or corresponding stained/unstained slides, concordance was observed in 84.4 % of cases. The majority of discordant results (57.1 %) consisted of samples classified as sperm negative by the microscopic examination that were positive by the immunochromatographic test; the opposite was observed in 42.9 % of cases.

3.3.3. DNA extraction

Of the 448 samples, 430 (96.0 %) underwent DNA extraction, which was standard in 61.9 % of cases and differential in 37.7 %, yielding sperm and epithelial fractions. Two standard extractions (0.5 %) were performed on material isolated by laser microdissection [20]. In general, the choice of DNA isolation technique reflected the results of the microscopic and immunochromatographic tests. The vast majority of samples in which the presence of spermatozoa was confirmed by microscopy were then subjected to differential extraction (92.5 %). The same was done in 74.5 % of samples with positive immunochromatographic results. Standard extraction prevailed (66.7 %) in samples that were sperm negative after microscopic examination and was the norm (93.8 %) in samples with a sperm negative immunochromatographic test. Results from a single laboratory, accounting for 73 (17.0 %) of the samples subjected to DNA isolation, reported consistent use of a "direct-to-DNA" approach, with standard DNA extraction applied regardless of the results of microscopic and immunochromatographic tests for the detection of sperm/semen.

3.3.4. DNA quantification

Of the total number of DNA extracts/fractions (n = 592), only 11.5 % - all from cases prior to 2016 - were not subjected to quantification. Real-time PCR methods, which simultaneously assess the concentration of total and male DNA and the degradation index, were used in 32.8 % of cases. RT-PCR assays discriminated only between total and male DNA in 31.3 % of cases. Blotting methods, which simply determine the total amount of DNA in the extract, were used in 25.2 % of cases, all before 2014.

Among DNA extracts/fractions analysed by RT-PCR methods, which allow the separate detection of total and male DNA, male DNA was observed in 71.1 % of samples that had shown the presence of spermatozoa by microscopic examination and in 17.4 % of those that had tested negative. Male DNA was also found in 77.5 % of the samples that

were positive in the immunochromatographic test for semen and in 35.8 % of the negative ones.

3.3.5. DNA typing results

In all but one of the 74 GT cases for which information was returned by the designated laboratory/expert, at least one of the DNA extracts obtained was considered potentially useful for PCR amplification. A total of 407 DNA extracts/fractions were subjected to PCR amplification targeting aSTRs (51.8 %), Y-STRs (4.9 %), or both aSTRs and Y-STRs (43.2 %). 341 aSTR profiles (88.1 % of samples amplified with aSTR kits) and 103 Y-STR haplotypes (53.3 % of samples amplified with Y-STR kits) were obtained. aSTR analysis generated single donor profiles in 66 % of the cases. Single donor profiles matched the victim in 79.6 % of the cases and a male subject in 20.4 % of the cases. The remaining profiles (34.0 %) were mixed. The vast majority of these were interpreted as mixtures containing the victim's DNA and one (73.3 %), two (14.7 %) or three (10.3 %) additional donors. Mixtures of two male subjects accounted for 1.7 % of all aSTR mixtures. Among the Y-STR haplotypes, 82.5 % were single-source. Haplotype mixtures with a minimal number of two and three contributors were 14.6 % and 2.9 %, respectively. Of all aSTR/Y-STR profiles/haplotypes, 14.4 % were highly incomplete with less than ten genotyped loci. The typologies of aSTR and Y-STR profiles are reported in detail in Supplementary Tables S1 and S2, respectively.

The percentage of DNA samples yielding a male DNA profile, in the form of single-source or mixed aSTR/Y-STR profiles/haplotypes, or both, is shown in Table 2. Data are presented for the total set of extracts and subsets of samples according to analytical strategy (direct-to-DNA, or traditional, i.e. performing either standard or differential extraction depending on spermatozoa/semen BFI results), BFI results and sample type. The proportion of male DNA results obtained from the standard extraction and the sperm and epithelial fractions of differential extractions are also given.

In total, 41.4 % of the samples yielded a male DNA profile. The proportion of samples yielding male DNA profiles was 52.1 % when using a direct-to-DNA approach and 39.2 % when adopting a traditional strategy. The success rate was almost identical when only sperm fractions from differential extracts were considered (51.2 %). Detection of male DNA profiles was also significantly more frequent in samples that were positive by microscopic examination of spermatozoa (Chi-square test, $p < 0.001$) and by immunochromatographic examination of semen (Chi-square test, $p < 0.001$). Considering the source of the sample, the detection of male DNA varied, ranging from 27.8 % of anal-rectal swabs to 72.7 % of skin swabs. Notable exceptions were condoms (100 %) and buccal swabs and subungual scrapings (0 %).

Characteristics of aSTR profiles showing male DNA contribution in the total set and subsets of samples according to extraction strategy, BFI results and sample type are summarised in Table 3.

The proportion of samples in which male DNA information was exclusively derived from Y-STR haplotypes is given in Table 4.

Table 2
Proportions of samples yielding a male DNA profile in the total set and in subcategories defined according to analytical strategy, BFI results for spermatozoa/semen and sample type. In the "Total" column, the presence of male DNA in the sperm and/or epithelial fraction of a given sample is counted as a single observation. Results separated by DNA extraction method are shown on the right. The absolute number of DNA extracts within each subcategory is given in parentheses. Results for intimate swabs and stained/unstained microscopic slides submitted as evidence were grouped according to anatomical location. Underwear also includes sanitary towels.

	Total	Standard extraction	Differential extraction (sperm fr.)	Differential extraction (epithelial fr.)
Total	41.4 % (n = 430)	32.1 % (n = 268)	51.2 % (n = 162)	26.5 % (n = 162)
Analytical strategy				
Direct-to-DNA	52.1 % (n = 73)	52.1 % (n = 73)	-	-
Traditional	39.2 % (n = 357)	24.6 % (n = 195)	51.2 % (n = 162)	26.5 % (n = 162)
BFI (spermatozoa/semen)*				
Microscopy +	64.2 % (n = 67)	20.0 % (n = 5)	64.5 % (n = 62)	22.6 % (n = 62)
Microscopy -	17.7 % (n = 96)	23.4 % (n = 64)	0 % (n = 32)	6.3 % (n = 32)
Immunochromatography +	78.4 % (n = 97)	69.6 % (n = 23)	78.4 % (n = 74)	40.5 % (n = 74)
Immunochromatography -	27.2 % (n = 195)	27.9 % (n = 183)	16.7 % (n = 12)	16.7 % (n = 12)
Specimen**				
Cervicovaginal	34.5 % (n = 142)	15.9 % (n = 63)	43.0 % (n = 79)	19.0 % (n = 79)
External genitalia	40.5 % (n = 42)	21.1 % (n = 19)	60.9 % (n = 23)	17.4 % (n = 23)
Ano-rectal	27.8 % (n = 18)	33.3 % (n = 9)	22.2 % (n = 9)	11.1 % (n = 9)
Oral	0 % (n = 20)	0 % (n = 14)	0 % (n = 6)	0 % (n = 6)
Skin	72.7 % (n = 11)	66.7 % (n = 9)	100 % (n = 2)	100 % (n = 2)
Subungual scraping	0 % (n = 8)	0 % (n = 7)	0 % (n = 1)	0 % (n = 1)
Underwear	53.0 % (n = 83)	41.4 % (n = 58)	76.0 % (n = 25)	40.0 % (n = 25)
Condom	100 % (n = 11)	100 % (n = 5)	100 % (n = 6)	100 % (n = 6)
Other clothing	46.8 % (n = 62)	44.8 % (n = 58)	25.0 % (n = 4)	75.0 % (n = 4)
Other objects	45.2 % (n = 31)	32.0 % (n = 25)	100 % (n = 6)	50.0 % (n = 6)

*Number of observations is > 430, since several samples were subjected to BFI by means of both microscopy and immunochromatography.
**Two samples of unknown biological source were not considered.

Table 3
The total number of observed aSTR profiles including male DNA is given in the "n" column. The percentage distribution of typologies of male aSTR DNA profiles is shown alongside: "Male DNA only" column includes both single source male DNA aSTR profiles and mixtures with only male contributors.

	aSTR profiles including male DNA		
	n	**Male DNA only**	**Mixtures with victim's DNA**
Total	166	31.3 %	68.7 %
Analytical strategy			
Direct to DNA	30	10.0 %	90.0 %
Traditional	136	36.0 %	64.0 %
DNA extraction method			
Standard	68	17.6 %	82.4 %
Differential sperm fraction	70	47.1 %	52.9 %
Differential epithelial fraction	28	25.0 %	75.0 %
BFI (spermatozoa/semen)*			
Microscopy +	39	33.3 %	66.7 %
Microscopy -	7	57.1 %	42.9 %
Immunochromatography +	82	35.3 %	64.7 %
Immunochromatography -	41	9.8 %	91.2 %
Specimen			
Cervicovaginal	34	26.5 %	73.5 %
External genitalia	15	53.3 %	46.7 %
Ano-rectal	5	20.0 %	80.0 %
Skin	9	55.6 %	44.4 %
Underwear	41	12.2 %	87.8 %
Condom	15	73.3 %	26.7 %
Other clothing	30	6.7 %	93.3 %
Other objects	17	64.7 %	35.3 %

*Number of male aSTR profiles is > 166, since several samples were subjected to BFI by means of both microscopy and immunochromatography

Table 4
For samples yielding a male DNA profile (absolute number is given in column "n") the proportions of cases where male DNA information was exclusively derived from Y-STR typing are shown.

	n	**Y-STR only**
Total	178	25.8 %
Analytical strategy		
Direct to DNA	38	21.1 %
Traditional	140	27.1 %
DNA extraction method*		
Standard	86	20.9 %
Differential sperm fraction	83	15.7 %
Differential epithelial fraction	43	34.9 %
BFI (spermatozoa/semen)**		
Microscopy +	43	37.2 %
Microscopy -	17	58.8 %
Immunochromatography +	76	28.9 %
Immunochromatography -	53	26.4 %
Specimen***		
Cervicovaginal	49	51.0 %
External genitalia	17	35.3 %
Ano-rectal	5	20.0 %
Skin	8	12.5 %
Underwear	44	27.3 %
Condom	11	0 %
Other clothing	29	0 %
Other objects	14	0 %

*Number of observations is > 178, since results of sperm and epithelial fractions in the same sample were counted separately.
** Number of observations is > 178, since several samples were subjected to BFI by means of both microscopy and immunochromatography.
***One sample of unknown biological source was not considered.

The percentage of samples subjected to direct-to-DNA analysis that yielded aSTR profiles without victim DNA contribution (10 %) was significantly lower than that observed for the traditional extraction (36.0 %) (Chi-square test, $p < 0.05$). Using the two different analytical approaches, the proportion of samples for which only Y-STR haplotyping was successful was 21.1 % and 27.1 %, respectively. Male aSTR-only profiles were significantly more frequent in the sperm fractions of the differential extracts (Chi-square test, $p < 0.001$), whereas the proportion of male DNA profiles obtained exclusively by Y-STR analysis was significantly higher in the epithelial fractions (Chi-square test, $p < 0.001$). With regard to BFI and the type of specimen, the only significant difference observed was in the proportion of male-only aSTR profiles between immunochromatography samples that had tested positive or negative for semen (35.3 % and 9.8 %, respectively) (Chi-square test, $p < 0.05$). Among samples taken from the body of sexual assault victims, the highest proportion of male-only aSTR profiles was observed in skin swabs (55.6 %).

Table 5
Percentages of samples yielding a male DNA profile and median TSI in hours (IQR) as a function of the presence/absence of assault-related activities according to medical records are given for the total set of samples and subcategories, together with Chi-square test and Mann-Whitney *U* test p-values, respectively.

Total		
Ejaculation	**male DNA**	$p > 0.05$
Yes (n = 119)	51.3 %	
No (n = 22)	31.8 %	
Condom	**male DNA**	**$p < 0.05$**
Yes (n = 13)	76.9 %	
No (n = 202)	44.6 %	
Bathing or showering	**male DNA**	**$p < 0.05$**
Yes (n = 150)	34.0 %	
No (n = 265)	47.9 %	
TSI	**hours**	**$p < 0.001$**
Male DNA (n = 174)	6 (IQR: 4–9)	
No male DNA (n = 237)	12 (IQR: 4–24)	
Victim's body*		
Ejaculation	**male DNA**	**$p < 0.05$**
Yes (n = 87)	48.3 %	
No (n = 16)	12.5 %	
Condom	**male DNA**	$p > 0.05$
Yes (n = 5)	40 %	
No (n = 152)	36.8 %	
Bathing or showering	**male DNA**	$p > 0.05$
Yes (n = 84)	29.8 %	
No (n = 159)	36.5 %	
TSI	**hours**	**$p < 0.001$**
Male DNA (n = 79)	4 (IQR: 4–8.5)	
No male DNA (n = 161)	10.5 (IQR: 4–17)	
Underwear		
Ejaculation	**male DNA**	$p > 0.05$
Yes (n = 24)	58.3 %	
No (n = 6)	83.3 %	
Condom	**male DNA**	-
Yes (n = 0)	-	
No (n = 38)	65.8 %	
Bathing or showering	**male DNA**	$p > 0.05$
Yes (n = 28)	46.6 %	
No (n = 52)	59.6 %	
TSI	**hours**	**$p < 0.05$**
Male DNA (n = 44)	6 (IQR: 4–9)	
No male DNA (n = 36)	8.5 (IQR: 5.5–24)	
Other clothes/objects		
Ejaculation	**male DNA**	-
Yes (n = 7)	71.4 %	
No (n = 0)	-	
Condom	**male DNA**	-
Yes (n = 8)	100 %	
No (n = 11)	72.7 %	
Bathing or showering	**male DNA**	**$p < 0.001$**
Yes (n = 37)	32.4 %	
No (n = 53)	71.7 %	
TSI	**hours**	$p > 0.05$
Male DNA (n = 50)	6 (IQR: 4–27)	
No male DNA (n = 40)	12 (IQR: 11.25–27)	

*Samples of unknown biological source were not considered

3.3.6. DNA results and medical history

The results of genetic testing were correlated with information from the medical records of GT cases that could be potentially relevant to the persistence and recovery of male DNA. The results are summarised in Table 5.

It could be seen that when ejaculation was reported, the percentage of samples taken from the victim's body yielding male DNA profiles (47.7 %) was significantly higher than in cases where ejaculation was not reported (12.5 %) (Chi-square test, $p < 0.05$). In general, the use of a condom was associated with a significantly higher rate of male DNA positive samples, but the result was driven by the excess of positive results in the other clothes/objects category, which included used condoms given by victims to SVS staff. When looking at the whole set of samples, male DNA was recovered at a significantly higher rate when the victim had not bathed or showered after the aggression. Curiously, this result appeared to be due to the high proportion of positive results from other clothes/objects rather than from samples taken from the victim's body or underwear, including sanitary towels. Male DNA was still obtained in 25 % of swabs collected from the external genitalia of victims who reported bathing or showering after the incident. The impact of bathing or showering on the chance of recovering male DNA from skin samples, which was in general rather high (72.7 %), could not be evaluated since all the victims from whom skin samples were collected did not report bathing or showering Finally, the average TSI was significantly shorter for samples yielding male DNA profiles in all categories except in other clothes/objects.

3.3.7. DNA comparisons

The results of DNA comparisons in GT cases are summarised in Fig. 3.

Of the 74 GT cases examined, 45 (60.8 %) yielded DNA profiles different from that of the victim, and 50 had a reference sample from at least one suspect. It was noted that the percentage of cases without suspect reference samples was higher for investigations assigned before (37.7 %) rather than after (19.0 %) the activation of BDN-DNA.

In 29 of the GT cases with suspect reference samples, DNA comparisons resulted in a match with at least one suspect. In eight of these cases, two suspects produced matches with evidence collected during FME, resulting in a total of 37 matches. A total of 147 matching DNA profiles were observed, consisting of: single-donor aSTR profiles (12.9 %); single-donor Y-STR haplotypes (38.1 %); aSTR mixture with likelihood ratio calculations supporting suspect contribution (40.8 %); Y-STR haplotype mixture including suspect alleles (8.2 %).

In total, 37 different DNA profiles were obtained that did not match either the victim or the suspect. Of these, 14 were from cases without suspect reference samples. Of the remaining 23 samples, 5 belonged to four cases in which the evidence collected during the FME produced exclusively male DNA profiles that did not match any of the suspects, the aggression was reported to have been committed by a single assailant, and the unknown male DNA profiles were obtained either from intimate samples (three cases) or from a used condom handed over by the victim (one case). The DNA results could therefore be considered as a relevant element of exculpation.

In 26 (35.1 %) cases, the designated laboratories/experts also received for examination samples collected outside the FME, typically objects and stains found at the crime scene. In 14 of these cases, this type of evidence led to useful aSTR/Y-STR profiles, and in five cases, it was the source of previously unobserved male DNA profiles, bringing the total percentage of cases with DNA profiles different from that of the victim to 50 (67.6 %). In three of these cases, the DNA obtained matched one (two cases) or two (one case) suspects, increasing the number of GT cases with at least one match to 32 (43.2 %) considering the whole period 2003–2023. Percentage of cases with a DNA match were 37.7 % and 57.1 %, respectively, for the periods before and after activation of BDNDNA. The remaining two cases resulted in two male DNA profiles of unknown origin.

Of the 40 male DNA profiles matching a suspect and 39 unknown male DNA profiles obtained from the samples collected during FME and from the associated crime scene, 13 and 15, respectively, were obtained after activation of BDN-DNA. However, only 3 and 4 DNA profiles respectively were uploaded to BDN-DNA (25.0 % overall). Male DNA uploaded to BDN-DNA consisted of Y-STR profiles in 57.1 % of the cases and of aSTR profiles in 42.9 % of the cases. Of the 21 DNA profiles that were not uploaded, 8 (3 matches with suspects and 5 unknowns) did not meet the quality standards for inclusion in BDN-DNA. For the remaining 13 DNA profiles (7 matches and 6 unknowns), there was no request from the judicial authority, which is required for inclusion in the BDN-DNA.

4. Discussion

The first element to emerge from the study is the extremely low proportion of cases (7.8 %) in which the judicial authority authorised or requested genetic testing of biological samples taken during the FME of sexual assault victims treated at the SVS centre in the period 2003–2023. This result is consistent with previous observations from the period before the implementation of BDN-DNA in the same specialist centre (7.6 % between 2003 and 2013 [11]), as well as from a similarly organised sexual assault support centre in northern Italy (6.9 % between 2006 and 2015 [12]). In contrast to other legal systems, where the main reason for untested sexual assault evidence (so-called sexual assault kits, SAKs) is backlog [21], i.e. material that has been sent to the laboratory but is awaiting analysis, it is evident that in Italy the vast majority of samples stored in hospitals remain unsubmitted and never undergo DNA testing. The issue of unsubmitted SAKs has been thoroughly investigated in the USA, with recent research showing that the proportion of SAKs submitted for testing ranges from 20 % to 60 % [22]. Although in the last year considered in this study (2023) the proportion of GT cases doubled (14.3 %), compared to pre-BDN-DNA standards, percentages remain below those observed even in extreme cases in the USA. As there is a lack of data on unsubmitted SAKs outside the USA, it would be useful to collect new data in the future to determine whether the situation in Italy is similar to that observed in other countries. This is particularly pertinent in Europe, where the European Union Prüm arrangement provides for the transnational exchange of DNA data, including BDN-DNA [23].

Some general criteria that may have guided the judicial authority in the selection of cases deemed worthy of DNA testing, and which may explain the overall limited proportion of genetic investigations, can be derived from the case study. First, cases with recent sexual contact appeared to be prioritised. The national guidelines for health professionals performing FME do not provide detailed information on the limits within which it is reasonable to collect biological samples from different anatomical sites of the victim [13]. The SVS internal operating protocol specifies the collection of samples up to a TSI of 240 h for cervicovaginal swabs, 120 h for ano-rectal swabs and 48 h for oral swabs. The maximum TSIs reported in the literature at which DNA testing can be expected to be successful vary widely [24–26]. In the present dataset, male DNA profiles were obtained from intimate swabs at a maximum TSI of 27 h, although this result may be biased by the under-representation of GT cases with a longer TSI. Also, samples taken from the victim's body and underwear which successfully yielded male DNA profiles had a significantly shorter average TSI than similar samples with no DNA results. In line with other international guidelines [27,28], GEFI has recently recommended limiting collection times to 120 h for cervicovaginal swabs, 72 h for ano-rectal swabs and 24 h for oral swabs [14]. Reducing the TSI limit for the collection of biological evidence will not have a direct effect on the absolute number of GT cases, but it may at least help to reduce the proportion of unsubmitted cases by excluding from the outset those that are unlikely to be useful and whose collection is therefore poorly justified [29,30]. In this way, it is possible to optimise the human, material and spatial resources available in hospitals for the collection and archiving of biological evidence. Most importantly, it spares victims from having to endure the complex

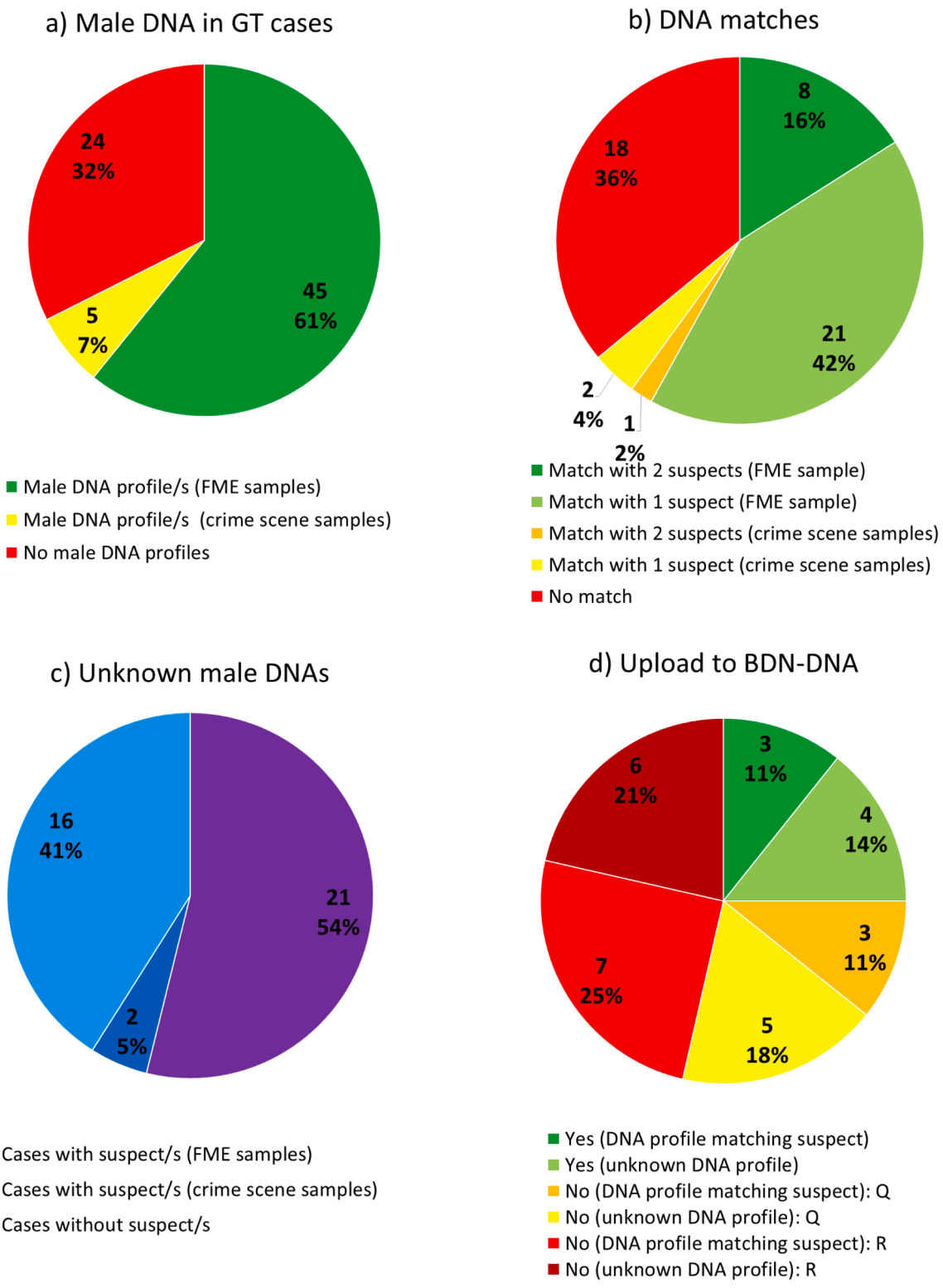

Fig. 3. Summary of DNA comparison results. DNA profiles obtained from samples collected during FME and other samples collected at the crime scene and sent to the laboratory are counted separately. Absolute and percentage values are given: a) GT cases (n = 74) categorised by observation of male DNA profiles; b) GT cases with suspect(s) reference samples (n = 50) categorised by DNA matches; c) unknown DNA profiles (n = 37) categorised by GT case type; d) upload to BDN-DNA of DNA profiles obtained in the period 2017–2023 (n = 29): Q indicates DNA profiles not included in BDN-DNA for quality reasons; R indicates DNA profiles not included in BDN-DNA for lack of a request from the judicial authority.

process of collecting biological evidence, which is stressful in itself and creates expectations that, if not met, can lead to feelings of abandonment and depression, with a consequent reluctance to seek further help [31].

Another prioritisation factor was identified in the significantly higher proportion of GT cases compared to NGT cases in which the perpetrator's ejaculation was reported by the victim and/or confirmed by the CS-UOGHT microscopic examination. The proportion of samples taken from the victim's body that tested positive for male DNA was significantly higher when ejaculation was reported in the medical record and, in general, male DNA profiling was significantly more successful in samples showing the presence of spermatozoa after microscopic examination and/or semen after immunochromatographic test results. However, it is important to emphasise that, in agreement with the literature, it was possible to obtain male DNA profiles in samples taken from the body of victims during FME, even when they denied ejaculation (12.5 % of cases) [32] or reported the use of a condom by the aggressor (40 %) [30], and in samples that were negative for spermatozoa after microscopic examination (17.7 %) [33,34] or for semen after immunochromatographic test (27.2 %) [35]. Excessive reliance on the medical history of reported ejaculation and the results of the microscopic search for spermatozoa could lead the judicial authority to dispense with genetic testing in such cases a priori, in the belief that its usefulness is limited. This is particularly true for protocols, such as the one in force at the SVS centre, which provide by default for microscopic examination of samples in the hospital and the inclusion of the results in the medical file, which is then sent to the judicial authorities in cases that can be prosecuted ex officio.

The results of preliminary BFI tests indicating the presence of semen can effectively guide subsequent laboratory investigations, e.g. the decision to use differential extraction. However, in recent years, a direct-to-DNA approach to sexual assault samples has been proposed, involving standard extraction and immediate amplification of aSTR/Y-STR, possibly supported by the results of male DNA quantification by RT-PCR [36], which has been shown to be highly effective in identifying male DNA profiles [37]. This was confirmed in the present study, where the proportion of samples yielding male DNA profiles in the case history of a single laboratory using a direct-to-DNA strategy (52.1 %) overlapped with that observed in the sperm fractions of DNA extracts (51.2 %) achieved by laboratories opting for a traditional approach in which either standard or differential extraction is performed according to BFI results. The future transition to a direct-to-DNA approach will eliminate the need for a preliminary microscopic search for spermatozoa, which, as previously highlighted, can be potentially misleading for the judicial authority.

Among the circumstances derived from the SVS medical record with potential effects on the persistence and recovery of male DNA, in contrast to what has been reported in other settings [38], bathing or showering did not appear to be a factor prioritised by the judicial authority when selecting cases for genetic testing. According to the SVS internal operational protocol, swabbing of external genitalia and skin is not recommended when bathing or showering is reported. However, it should be noted that male DNA was found in 25 % of external genital swabs collected despite the victim reporting that they had bathed or showered after the sexual assault. Although bathing or showering should not be considered a valid reason for not submitting a sample to the forensic laboratory, the absence of these activities was associated with a higher recovery of male DNA profiles, in line with previous research [30]. However, this was mostly seen in evidence other than swabs taken from the victim's body and underwear, such as clothing and other items collected during FME, and could be partly due to the presence of background DNA unrelated to the crime [30]. Accordingly, clothing other than underwear was the type of item with the highest rate of mixed aSTR profiles containing the victim's DNA and that of up to three additional contributors.

Returning to the issue of unsubmitted swabs, further possible explanations include: lack of request/authorisation from the victim to release the kit for testing [31]; suspected perpetrator not yet identified [39]; identified perpetrator in a known sexual relationship with the victim, so the evidence collected may not be considered probative in "consent" cases where the suspect claims the sexual contact was consensual [40]; doubts about the credibility of the victim, especially in the absence of injuries suggesting they did not fight the perpetrator [41].

In the present study, the occurrence of conditions, including group violence, leading to ex officio prosecution, and thus making the victim's request to initiate an investigation unnecessary, was significantly more common in GT cases. Nevertheless, 83 % of NGT cases were also prosecuted ex officio, suggesting that the absence of a victim's request for a prompt investigation may have only a minimal impact on the high proportion of unsubmitted SAKs.

With regard to the type of suspect, the proportion of assaults perpetrated by persons previously unknown to the victim (strangers) was significantly higher in GT cases (61.1 %) than in NGT cases (42.3 %). The appropriateness of prioritising cases involving strangers over non-stranger perpetrators has been debated. While some authors have recommended this approach because DNA evidence may be the best and possibly only means of identifying and prosecuting the suspect [42], it has been shown that stranger and non-stranger SAKs are equally likely to produce criminal database hits [43]. As rapists are often repeat offenders, victimising different types of victims within and outside intimate partner relationships [44], genetic analysis and inclusion of non-stranger SAKs in the BDN-DNA may be useful to detect seriality by linking DNA profiles across cases [39]. In the Italian scenario, another element that should stimulate genetic investigations and the inclusion of non-stranger perpetrator DNA profiles in the BDN-DNA is the extreme delay in the processing of reference samples from arrested and detained individuals, which by law are included in the BDN-DNA by default. Apparently, only 10 % of these individuals' DNA profiles have been included in the BDN-DNA so far [17], making case-derived DNA profiles the main tool for linking sexual offences and identifying offenders at present.

With the activation of BDN-DNA in 2017, sexual assaults where the perpetrator has not yet been identified, making direct comparison of DNA profiles impossible, will also be potentially relevant for genetic investigation. Although data on the Italian BDN-DNA are still limited [17], the benefits in combating sexual offences are evident in studies from other countries that have previously implemented a criminal DNA database, such as the Netherlands [45], Australia [46] and the USA [47]. It was therefore hoped that introducing BDN-DNA would halt the steady decline in GT cases observed at the SVS centre since 2005, which culminated in 2017 when no samples were analysed. This negative trend could possibly be explained by the gradual disenchantment of the judicial authority, after an initial infatuation with genetic investigation tools, as a consequence of results perceived as unsatisfactory (<40 % of cases yielding a match) and of the disproportionately high costs. In part, however, the discouraging results reflect inappropriate use of genetic testing. For example, prior to the availability of BDN-DNA, the usefulness of genetic testing in the absence of a suspect appears questionable, as was the case in 37.3 % of GT cases investigated between 2003 and 2016. Nevertheless, given that this is the first study to examine trends in DNA analysis in sexual assault cases following the introduction of the Italian BDN-DNA, it was surprising to discover that the proportion of GT cases without a suspect decreased to 19 % between 2017 and 2023.

The proportion of GT cases yielding DNA profiles different from that of the victim (60.8 %) was consistent with previous studies [48,49]. However, only 25 % of DNA profiles obtained after 2017 were uploaded to the BDN-DNA database. Even when accounting for differences in national legislation regarding the criteria for including a DNA profile in a criminal database, this percentage is lower than expected based on previous studies of samples collected in cases of sexual assault (47–82 %). [43,50].

Of the DNA profiles that were not uploaded, 38.1 % had quality limitations (partial profiles, mixtures) that prevented their inclusion in

the BDN-DNA in accordance with current legislation. On this point, it could be argued that a direct-to-DNA analytical strategy, which omits sperm DNA separation by differential lysis, increases the proportion of mixed aSTR profiles that include the victim's DNA (90 % in the present case history, compared to 64.0 % for the traditional approach) and, as a consequence, may not be suitable for BDN-DNA. According to BDN-DNA regulations, mixed STR profiles cannot be uploaded for international DNA exchange, unless a major component with peak height ratio of at least 3:1 is observed across all heterozygous loci, in which case the major DNA profile can be considered equivalent to single source. However, this problem can be effectively circumvented thanks to the fact that Italian BDN-DNA also includes Y-STRs, with 80 % of direct-to-DNA samples yielding single-source Y-STR haplotypes. Although Y-STR matches are not individual-specific [51], they enable to identify a candidate list of offenders whose aSTR profiles can be then directly compared with aSTR mixtures obtained from the case, if available, through probabilistic genotyping methods [52]. It must be noted that the percentage of cases in which male DNA results were exclusively derived from Y-STRs and therefore a posteriori evaluation of mixed aSTR profile would not be possible was smaller for the direct-to-DNA approach (21.1 %) compared to the traditional analytical strategy (27.1 %).

The remaining 61.9 % of DNA profiles, although of high quality, were not included in BDN-DNA due to the lack of a request from the judicial authority. The criteria that informed the decision not to upload these DNA profiles are unclear, as almost equal proportions of identified and unidentified male DNA profiles were uploaded or not uploaded to BDN-DNA. This may reflect a lack of awareness on the part of the Italian judicial authorities of the operation and potential of BDN-DNA, despite the great economic and organisational effort required to feed the system, which includes ISO/IEC 17025 accreditation of the laboratories and advanced information technologies to ensure the functionality of the database and the protection of the privacy of the data stored. A recent publication by Luparia et al. [16] showed that only ~80 % of Italian public prosecutors have ever requested the upload of a DNA profile to BDN-DNA and that only ~50 % of them do so systematically or at least several times a year. Percentages are even lower for judges (~10 % and ~30 %, respectively). To increase awareness, it seems desirable that, as has happened in other nations that have recently introduced a criminal DNA database [53], the number of scientific studies regarding the performance of BDN-DNA also grows rapidly in Italy. Having up-to-date public data on the number and type of samples collected, and the number of matches and investigations aided, can help identify and resolve critical issues that emerge in the early stages of implementation, and optimise operating procedures.

Among the factors influencing the judicial authority's decision to order or not to order genetic testing in sexual assault cases, consideration of the victim's credibility was also mentioned [41]. Contrary to what might be expected, the proportion of victims without personal injuries whose samples were genetically tested (46.7 %) was significantly higher than in the NGT comparison group (34.6 %). It is known that many women who report recent sexual aggressions have no evidence of genital and extra-genital injuries, so this does not rule out sexual assault [54]. Furthermore, tonic immobility (freezing) is well described as a common trauma response in rape victims [55]. In this case, it is possible to hypothesise that the use of DNA testing is privileged given the need to corroborate the victim's story in the absence of signs of trauma that could indicate that the sexual act was non-consensual [56].

A final aspect that emerges from the SVS case history 2003–2023 and that should not be overlooked is the importance of DNA testing not only as a tool for identifying sexual offenders, but also as an element of exoneration for individuals who may have been wrongly accused [57]. In 5.4 % of single perpetrator assaults investigated, male DNA profiles obtained from intimate samples from the victim did not match the suspect, providing strong evidence against his involvement in the case, as long as other recent consensual sexual activity by the victim could reasonably be excluded [26].

5. Conclusions

The case history of over 1000 patients who sought help at a specialised Italian hospital centre for victims of sexual assault shows that the vast majority of samples collected during FME remain unsubmitted and never undergo genetic testing. One possible strategy for improvement could be to update national and internal hospital guidelines to rationalise the sampling of victims' bodies: by focusing on cases with a lower TSI and a higher probability of success in genetic testing, it would be possible to make more effective use of the human and financial resources allocated and reduce the risk of creating false expectations and further negative experiences for patients.

The results of the study may also prove useful in raising the awareness of the judicial authority of the merits and limitations of elements that can be derived from the medical records of sexual assault victims and that can be used as selection criteria to identify the cases to be subjected to genetic testing, such as reported ejaculation, condom use, bathing or showering and the results of microscopic examination of spermatozoa.

Finally, it is hoped that the results obtained can make a useful contribution to national criminal justice policies against sexual violence, in particular by helping to better understand the potential of BDN-DNA, which appears to be under-utilised at present.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2025.103377.

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