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# Advantages of Radiofrequency Echographic Multi Spectrometry (REMS) in evaluating osteoporosis in the rural area of Sannio: A clinical and economic analysis of a free screening program

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ABSTRACT

*Objective:* Osteoporosis (OP) is a major public health issue in countries with high life expectancy such as Italy, particularly affecting rural populations with limited access to diagnostic and specialist services. This study aimed to investigate OP risk factors, disease awareness, and healthcare experiences in an Italian rural community, and to evaluate the clinical and economic impact of Radiofrequency Echographic Multi Spectrometry (REMS) as a radiation-free diagnostic tool.

*Methods:* A free, touring OP screening program was conducted in the rural area of Sannio, southern Italy. Participants underwent semi-structured interviews, fracture risk assessment using the FRAX tool, REMS-based bone evaluation, and biochemical analyses to assess calcium–phosphate metabolism and secondary causes of OP.

*Results:* A total of 347 participants were enrolled in the screening program. Osteoporosis was diagnosed in 37.8% of subjects, while 54% were classified as having osteopenia. Participants reported high satisfaction with the screening initiative. Economic analysis demonstrated that REMS was associated with significant cost savings compared with DXA, with an average reduction of €35.7 per patient, while maintaining diagnostic reliability in both female and male populations.

*Conclusion:* REMS represents a feasible, accurate, and cost-effective diagnostic technology for early osteoporosis detection in rural healthcare settings. Its implementation within the Italian National Health Service (NHS) may improve access to osteoporosis care, reduce health disparities, and support early prevention strategies in underserved populations.

## 1. Introduction

Osteoporosis (OP) and osteopenia affect more than half of adults aged over 50 years, representing a major public health issue and a leading cause of disability and fracture-related morbidity worldwide.[1,2] It is defined as a systemic condition characterized by micro-architectural bone deterioration resulting in a reduced bone mineral density (BMD).[3,4] OP represents a significant economic burden in Europe with an estimated cost of about € 56.9 billion in 2019 (mostly related to incident fractures),[5,6] and it costed approximately € 2.2 billion to the Italian national health system (NHS), about 80% of which was due to hospitalizations.[7] Moreover, Italy is one of the countries with the highest

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percentage of elderly people in the general population.[8,9] In this perspective, OP and fragility fractures represent a leading health-cost driver for Italian [7,8,10–12], since incidence and costs of hip fractures in Italy are comparable with those of acute myocardial infarctions, with an average cost of € 13,500 per patient.[10]

It has been reported extensively how people living outside major urban centers experience higher incidence of OP rates compared with city residents, along with limited awareness, reduced access to screening, reduced referral to OP specialists, and suboptimal treatment[13–18]. Sannio, the region examined in this study, covers a predominantly rural area of approximately 2071 $km^2$ and, as of 2011, has a population of 284,900 inhabitants.[19] It is located in the southern Italy, in the administrative area of the city of Benevento, and it is characterized by the absence of highways or fast long-distance routes and the presence of only one hospital, which may be up to 90 min away from several residential centers. Sannio has a high percentage of older people, since approximately 10.1% of the population is 75 years old or older, and the Ageing Index, defined as the ratio between the elderly population (65 years and over) and the younger (0-14 years), is 150.8[19],[20].

REMS is an innovative technology for OP assessment, based on the analysis of the raw unfiltered ultrasound signals acquired during an echographic scan of lumbar spine and/or femoral neck.[21] Several single and multicenter studies have validated the diagnostic accuracy of REMS compared to DXA[22–24]. Moreover, it offers distinct advantages, including high diagnostic precision, rapid execution, ease of use, portability, and the absence of radiation exposure.[21,23] Since DXA is not easily available in rural areas, and reimbursement for DXA was conditional and varied depending on the patient's condition,[5] REMS appears particularly suitable for large-scale or mobile screening programs in geographically disadvantaged or low-income areas.[21,25]

Therefore, this study aims to explore OP risk factors, disease awareness, experiences, and preferences for care among people living in the low-income, rural area of Sannio, evaluating the feasibility and the clinical and economic impact of REMS in this context. In this report, the results of an epidemiological campaign carried out between September 2022 and October 2024, are described.

## 2. Materials and methods

Data deriving from a touring screening campaign promoted by local non-profit associations (22 screening days in 19 villages) is reported in this paper. The study was approved by the Internal Review Board of the “San Pio Hospital” of Benevento. Adult subjects of both sexes aged ≥40 years were recruited on a voluntary basis. Participants were also recruited via advertisements at local community associations, general practitioner's offices or social groups. The evaluation was conducted in a mobile ambulatory van, with a staff composed of a physician and a technician.

All recruited subjects underwent:

- ***REMS Measurement:*** Bone mineral density (BMD) was assessed using REMS technique (Echolight S.p.A., Lecce, Italy). Measurements were performed at the lumbar spine (L1–L4) and left proximal femur using a convex probe operating at 3.5–5.0 MHz. Subjects were examined in the supine position, and each acquisition lasted approximately 60–90 s. The automatic quality control algorithm excluded scans with artifacts or poor signal-to-noise ratio. All measurements were conducted by trained operators using the standard manufacturer's protocol and calibration settings, ensuring reproducibility and diagnostic precision.
- ***Semi-Structured Interviews:*** A complete medical history collection was performed immediately after the REMS procedure. The interviewer investigated OP risk factors (including FRAX™ calculation), lifestyle, comorbidities, secondary causes of OP (i.e., hyperthyroidism, nephrolithiasis, celiac disease, inflammatory bowel diseases, rheumatoid arthritis), previous OP fractures, therapies and awareness of the disease. Once the individual risk of fracture was established, the medical staff handed out counselling for the prevention of OP and fragility fractures, then a Satisfaction Survey was conducted.
- ***Blood and Urinary Tests:*** The physician prescribed blood and urine analysis in order to assess calcium-phosphate metabolism and exclude secondary causes of OP. The patients performed examinations after the screening and e-mailed the results to the physician. The blood analysis tests were: ESR, serum parathyroid hormone (PTH), thyroid hormones, protidemia; calcemia; phosphataemia; total alkaline phosphatase (ALP) and bone isoenzyme; creatininemia; Serum 25-OH-vitamin D; celiac disease antibodies. The urinary tests were: calciuria within 24 h; phosphaturia within 24 h.

After the obtainment of an informed consent, data were anonymously reported into an electronic database to allow pooled analysis.

## 3. Results

***Study group:*** A total of 347 subjects accessed the screening program. The demographic features of this population are represented in Table 1.

**Table 1**
Demographic details and OP risk factors of screening participants.

| | | All | Females | Males |
|---|---|---|---|---|
| Age (years) | mean ± SD | 62.4 ± 22.3 | 63.1 ± 21.8 | 58.7 ± 24.5 |
| Sex | n (%) | 347 (100%) | 322 (92.8%) | 25 (7.2%) |
| Menopause | | - | 277 (86%) | - |
| Family history[a] | n (%) | 245 (70.6%) | 230 (71.4%) | 15 (60.0%) |
| Education n (%) | Did not complete high school | 128 (36.9%) | 121 (37.6%) | 7 (28.0%) |
| | High school completion | 158 (45.5%) | 147 (45.6%) | 11 (44.0%) |
| | Diploma/ qualification | 50 (14.4%) | 45 (14.0%) | 5 (20.0%) |
| | Higher degree | 11 (3.2%) | 9 (2.8%) | 2 (8.0%) |
| Alcohol consumption n (%) | None | 80 (23.1%) | 76 (23.6%) | 4 (16.0%) |
| | <1 SD per day | 165 (47.6%) | 152 (47.2%) | 13 (52.0%) |
| | 1-2 SD per day | 61 (17.5%) | 55 (17.1%) | 6 (24.0%) |
| | >2 SD per day | 41 (11.8%) | 39 (12.1%) | 2 (8.0%) |
| Smoking habit n (%) | Never | 216 (62.2%) | 205 (63.7%) | 11 (44%) |
| | Former | 83 (24.0%) | 75 (23.3%) | 8 (32%) |
| | Current | 48 (13.9%) | 42 (13%) | 6 (24%) |
| Exercise n (%) | Sedentary | 201 (57.9%) | 186 (57.8%) | 15 (60.0%) |
| | Non sedentary | 146 (42.1%) | 136 (42.2%) | 10 (40.0%) |
| Comorbidities[b] n (%) | 0 | 136 (39.2%) | 124 (38.5%) | 12 (48.0%) |
| | 1 | 114 (32.9%) | 106 (33.0%) | 8 (32%) |
| | 2 | 67 (19.3%) | 64 (19.8%) | 3 (12.0%) |
| | ≥3 | 30 (8.6%) | 28 (8.7%) | 2 (8.0%) |

[a] Family history included presence of parental vertebral or hip fracture or parental diagnosis of osteoporosis.

[b] Comorbidities included: malabsorption (coeliac disease or inflammatory bowel disease), chronic kidney or liver disease, hyperthyroidism, hyperparathyroidism, inflammatory arthritis, obesity body mass index >30, underweight body mass index <18.5, epilepsy, diabetes (both type 1 or type 2), glucocorticoid use for 3 months or longer, early menopause before age 45 years.

There was a major female representation (n = 322, 92.8%) with a female to male ratio of about 13:1. Mean subject age was 62.4 ± 22.3 years (range 40-101); specifically, the mean age for females was 63.1 ± 21.8 years (range 41–101), while for males it was 58.7 ± 24.5 years (range 40–95). Amongst female participants, 277 (86%) were postmenopausal, and 80 (28.8%) of them had a history of gynecological or oncological ablative surgery.

Overall, 315 participants (90.8%) had previously undergone an OP screening test, and 131 (37.8%) already had a confirmed diagnosis of OP.

Most attendants had completed high school (n = 158, 45.5%), but a significative percentage of patients did not (n = 128, 36.9%) and only a little minority had a higher degree (n = 11, 3.2%).

***Bone status according to REMS:*** REMS results are represented in Table 2. Lumbar Spine ($L_1$-$L_4$) REMS results showed normal bone status in 30 patients (8.6%), osteopenia in 186 (53.6%), and OP in 131 (37.8%). Left Proximal femur REMS showed normal bone status in 27 patients (7.8%), osteopenia in 189 (54.4%), and OP in 131 (37.8%).

In the female subgroup (n = 322) Lumbar Spine ($L_1$-$L_4$) REMS results showed normal bone status in 28 patients (8.7%), osteopenia in 173 (53.7%), and OP in 121 (37.6%). Left Proximal femur REMS were overlapping, showing normal bone status 28 patients (8.7%), osteopenia in 173 (53.7%), and OP in 121 (37.6%).

In the male subgroup (n = 25) Lumbar Spine ($L_1$-$L_4$) REMS results showed normal bone status in 2 men (8%), osteopenia in 13 (52%), and OP in 10 (40%). Left Proximal femur showed normal bone status in 2 patients (8%), osteopenia in 14 (56%), and OP in 9 (36%).

Overall, lumbar and femoral REMS assessments revealed a similar bone status distribution in both females and males, with over half of the patient showing osteopenia and about than one-third classified as osteoporotic.

***Prevalence of risk factors:*** We investigated a past or family history of osteoporotic fractures. When asked if one of their parents had a diagnosis of OP or had shown signs of OP, such a major fracture occurred in absence of an efficient trauma (fragility fracture), 245(70.6%) of the interviewees answered "yes".

The majority of the sample reported an alcohol intake <1 standard drink (SD)/day (n = 165, 47.6%), while 23.1% (n = 80) had no alcohol intake and only 11.8% (n = 41) of the participants had more than 2 SD/day.

As far as smoking habit is concerned, non-smokers were the most represented category (n = 216, 62.2%), while prior or current smokers represented respectively 24% (n = 83) and 13.9% (n = 48) of the sample. According to gender, females showed a similar proportion (63.7% of non-smokers versus 36.3% of former/current smokers), while in the male subgroup smokers (56%, n = 14) outnumbered non-smokers (44% n = 11).

As regards physical exercise, the study population was sedentary: 201 participants (57.9%) engaged in <75 min of moderate or <35 min of vigorous physical activity per week. Underweight (body mass index <18.5), a known risk factor for OP, was found in 24 people (6.9%).

Chronic kidney disease (CKD), hyperparathyroidism, inflammatory arthritis, celiac disease and inflammatory bowel diseases (IBD) were assessed pooled together under the name of "Secondary causes of osteoporosis".

As a whole, secondary OP was found in 22,5% of the subjects (n = 78, 6 males and 72 females). The most common causes were inflammatory arthritis, found in 33 patients, CKD, found in 26 patients; and malabsorption (Celiac disease and IBD) found in 23 patients. The relatively high incidence of arthritis in the screened population could be a bias due to the fact that people living with this condition have a higher awareness of OP and actively participate in the free screening. Moreover, 21 people (6%) had reported to have taken glucocorticoids for at least 3 consecutive months, mostly for inflammatory conditions such as arthritis or IBD.

We finally assessed the mean 10-year fracture risk through FRAX algorithm. Medium FRAX score was 24.3% ± 10.9. A sub-analysis conducted by age group showed how FRAX increase with age: in the group 40-60 years old mean FRAX was 23.5% ± 10.9 (n = 155); in the group 61-90 years old mean FRAX was 25.0% ± 10.9 (n = 162); in the group ≥91 years old mean FRAX was 26.2% ± 11.4 (n = 30).

***Therapies:*** In a second phase, after REMS assessment, FRAX calculation and screening for secondary causes of OP, we prescribed pharmacological, behavioral and eventually rehabilitation therapies. In Italy, prescription of OP pharmacological treatment is based on a therapeutic algorithm endorsed by AIFA (Italian Medicines Agency) Note 79[26],[27]. AIFA's guideline establishes the criteria for the Italian NHS to reimburse drugs for OP treatment, defining eligibility based on fracture risk, primary or secondary prevention of fragility fractures, and lists the specific drugs available for reimbursement in each scenario. Based on this algorithm, the following therapies were prescribed: 10 patients (2.88%) did not receive pharmacological therapy; in 65 (18.73%) patients received only Vitamin D3 supplementation; 123 (35.45%) patients with Bisphosphonates and Vitamin D3; 111 (31.99%) patients were treated with Denosumab and Vitamin D3; finally, 38 women were treated with Romosozumab and Vitamin D3. By the time the study was conducted, Abaloparatide was still not available for prescription.

***Patients awareness and perspective:*** Overall, patient satisfaction was very high: 316 participants (91.07%) thought the screening tour was useful. OP awareness was very high, especially among women, and only 10 participants (2.9%) underwent screening solely because it was offered free of charge. Thirty-one participants (8.93%) were not satisfied: this was mainly due to bureaucratic difficulties in obtaining prescriptions or follow-up examinations.

**Table 2**
REMS results.

| All Participants (n = 347) | | n (%) | T-score mean ± SD | Z-score mean ± SD |
|---|---|---|---|---|
| Lumbar Spine (L1-L4) | Normal | 30 (8.6%) | −0.82 ± 0.43 | −0.85 ± 0.63 |
| | Osteopenia | 186 (53.6%) | −2.25 ± 2.06 | −2.16 ± 2.34 |
| | Osteoporosis | 131 (37.8%) | −3.45 ± 1.86 | −3.82 ± 1.68 |
| Left Proximal Femur | Normal | 27 (7.8%) | −0.93 ± 0.64 | −0.89 ± 058 |
| | Osteopenia | 189 (54.5%) | −2.38 ± 1.96 | −2.54 ± 1.84 |
| | Osteoporosis | 131 (37.7%) | −3.32 ± 1.28 | −3.56 ± 1.56 |
| **Female Participants (n = 322)** | | **n (%)** | **T-score mean ± SD** | **Z-score mean ± SD** |
| Lumbar Spine (L1-L4) | Normal | 28 (8.7%) | −0.82 ± 0.43 | −0.85 ± 0.63 |
| | Osteopenia | 173 (53.7%) | −2.25 ± 2.06 | −2.16 ± 2.34 |
| | Osteoporosis | 121 (37.6%) | −3.45 ± 1.86 | −3.82 ± 1.68 |
| Left Proximal Femur | Normal | 25 (7.8%) | −0.93 ± 0.64 | −0.89 ± 0.58 |
| | Osteopenia | 173 (53.7%) | −2.38 ± 1.96 | −2.54 ± 1.84 |
| | Osteoporosis | 121 (37.6%) | −3.32 ± 1.28 | −3.56 ± 1.56 |
| **Male Participants (n = 25)** | | **n (%)** | **T-score mean ± SD** | **Z-score mean ± SD** |
| Lumbar Spine (L1-L4) | Normal | 2 (8.0%) | −0.82 ± 0.43 | −0.85 ± 0.63 |
| | Osteopenia | 13 (52.0%) | −2.25 ± 2.06 | −2.16 ± 2.34 |
| | Osteoporosis | 10 (40.0%) | −3.45 ± 1.86 | −3.82 ± 1.68 |
| Left Proximal Femur | Normal | 2 (8.0%) | −0.93 ± 0.64 | −0.89 ± 0.58 |
| | Osteopenia | 14 (56.0%) | −2.38 ± 1.96 | −2.54 ± 1.84 |
| | Osteoporosis | 9 (36.0%) | −3.32 ± 1.28 | −3.56 ± 1.56 |

This table shows the distribution of bone mineral density categories evaluated with REMS in all participants, in female and male subgroups.

### 3.1. Cost-benefit

A recent paper conducted a cost-minimization analysis (CMA) to compare the direct costs for the Italian NHS between REMS and DXA, assuming that the two technologies have equivalent (or at least comparable) clinical outcomes in diagnostic terms.[28] Overall, in the base-case scenario, the adoption of REMS is associated with an average saving for the Italian NHS of €40.0 per patient (with an estimated savings range between approximately €27.6 and €71.5) compared with DXA. The authors indicate that these savings derive from the fact that REMS requires fewer resources (personnel, equipment, testing) in routine clinical practice in Italy compared with the DXA scenario.[28]

We utilized the same approach to calculate savings for the NHS when performing REMS compared to DXA in the rural population of Sannio. According to the prices reported in the cost sheets of San Pio Hospital in Benevento and the ASL of the Benevento district, which are the closest hospital and local health service providers in the Sannio area, professional service costs (healthcare professionals and administrative staff) were €30.00 for REMS and €49.20 for DXA. The cost for instrumental diagnostics and laboratory testing (specifically: complete blood count, serum calcium, serum phosphate, 25-hydroxy-vitamin D, PTH, and renal function tests) was €45.10 for REMS and €75.60 for DXA. The cost of consumables (i.e., gloves, medical bed sheets, gel) was not considered in the present analysis due to the very limited quantities used for both examinations (Fig. 1).

Taken together, these cost components indicate that the use of REMS is associated with a mean saving for the NHS of €35.7 per patient (with a total saving of €17,245.90 for the 347-screened cohort). This estimate is consistent with the savings reported in the CMA mentioned above.

Moreover, a possible savings range of €5 to €150 was calculated. This broader range reflects real-world conditions in the Sannio area: since the region is often underserved by the NHS, waiting lists for DXA are long, and the hospital is difficult to reach due to distance and poor transportation. As a result, many patients undergo DXA in private facilities at their own expense. Given that the average private DXA price in the area is 75.23 euros, this leads to a potential savings range extending to 150 euros (Table 3).

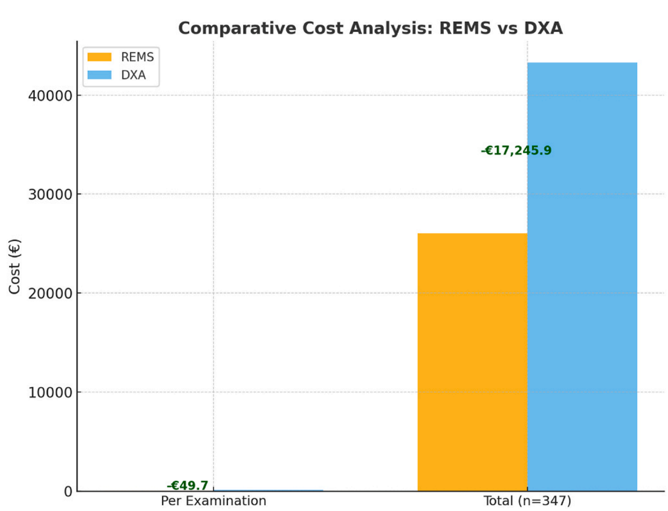


**Fig. 1.** Comparative cost analysis per examination and total cost for the study cohort
Comparison of mean per-examination and total costs between Radiofrequency Echographic Multi Spectrometry (REMS) and Dual-energy X-ray Absorptiometry (DXA). The analysis is based on Ministry of Health tariffs and data from Borsoi et al., *Glob Reg Health Technol Assess* 2023. REMS shows an average per-examination cost reduction of €49.7 and a total saving of €17,245.9 for the 347-screened cohort.

**Table 3**
Cost–Benefit Analysis: REMS vs DXA.

| Cost Component (€) | REMS | DXA | Δ |
|---|---|---|---|
| Professional costs | 30.0 | 49.2 | −19.2 |
| Instrumental examination + laboratory tests | 45.1 | 75.6 | −30.5 |
| Total cost per examination | 75.1 | 124.8 | −49.7 |
| Total cost for 347 subjects | 26,059.7 | 43,305.6 | −17,245.9 |

**Interpretation**: Under current economic conditions, REMS demonstrates a substantial cost advantage compared with DXA, leading to an average saving of €35.7 per patient (ranging between €5 and €150), and a total saving of approximately €17,200 for the 347 screened participants. This cost reduction is mainly attributable to lower instrumental and professional costs, as well as simplified logistics and absence of ionizing radiation.

## 4. Discussion

OP is a socially relevant condition whose incidence increases with age, affecting a large percentage of the elderly population in countries with long life expectancies.[3,29] Management of OP and its complications (fragility fractures) represent an emerging economic burden, particularly in countries with an ageing population like Italy[7,11,29–31]. This burden can be higher in rural and low-income areas, where access to DXA and OP specialists is difficult for both logistics and economic reasons.[14,16,18] This study offers valuable insight into the potential of REMS as a practical and economically sustainable solution for OP screening in rural communities.

In our study, 37.8 % of the subjects were osteoporotic according to REMS T-score values, while about 54% were osteopenic. The use of quantitative ultrasound for OP screening was already assessed in a large epidemiological campaign conducted via a mobile van carried out in the main Italian cities, in the so called “BoneTour”.[31] This study was carried out in the general population in order to identify “at-risk” individuals, but excluded rural areas, where DXA is not easily available and OP may be underdiagnosed: the high prevalence of OP observed in the rural population of Sannio (37.8% versus 18% reported in the “BoneTour” screening) confirms previous findings that rural residents face greater diagnostic and therapeutic inequities than their urban counterparts.[14,16,17] However, the magnitude of these findings should be interpreted with caution, as the study population was self-selected, potentially overestimating disease prevalence and limiting generalizability to the wider rural population. Indeed, in our sample, there was a high percentage of people suffering from inflammatory arthritis, maybe reflecting a higher disease awareness in this subgroup and a greater participation in this free public health intervention.

The majority of the sample (92.8 %) was composed by women, and this reflects the fact that in the collective opinion osteoporosis is still seen as a threat only for women, especially because of the estrogens drop that occurs during menopause: as proof of this, 86% of female participants were postmenopausal, and about 90% had undergone previous OP screening test. Males represented a small subgroup in the study, even if risk factors, such as smoke habit, are more incident in the male populations. Interestingly, males participating in the screening program were younger, maybe reflecting a higher awareness in younger people.

While REMS demonstrated high feasibility and diagnostic reliability[21–23], direct comparison with DXA was not performed on the study group. Therefore, although REMS has shown equivalence to DXA in other studies,[21] this specific dataset cannot definitively confirm diagnostic concordance, even if the percentage of people with OP with REMS evaluation is comparable with the percentage of people having a previous diagnosis of OP.

Additionally, the high satisfaction rate (>90%) and the big participation—though encouraging—may reflect a “gratification bias,” as participants received a free screening and personal medical feedback, which could have influenced their responses positively. Nonetheless, satisfaction also highlights the value of community-based healthcare initiatives in low-income areas with limited medical infrastructure.

Moreover, the organization of the screening program within local communities played a pivotal role in participation rates. In rural settings, social cohesion and the presence of trusted institutions often facilitate adherence to public health interventions, while urban populations benefit from proximity to healthcare facilities but frequently show lower adherence to preventive programs due to time constraints or lack of community engagement.[32] This sociocultural difference underscores the importance of tailoring health strategies to the social structure of rural environments.

Economically, REMS showed a mean saving of €35.7 per patient compared with DXA, confirming previous analyses that identified ultrasound-based technologies as cost-effective and accessible for primary prevention.[28,33–36] Yet, in a real world scenario, NHS do not serve properly rural areas leading to an increase in costs for low income areas which rely on private healthcare.

Moreover, the real-world sustainability of REMS screening programs depends also om operator training and equipment maintenance costs which were not evaluated in this study. Thus, while promising, the projected economic benefit requires further validation through health-economic modeling and multicentric data. Despite these limitations, several strengths reinforce the reliability of the findings: the study's community-based approach, the integration of clinical, biochemical, and social dimensions, and the inclusion of a large rural sample previously underserved by traditional healthcare systems.

These findings support REMS as a feasible, accurate, and cost-effective technology for the early diagnosis and prevention of osteoporosis in rural healthcare settings. Although the results must be interpreted prudently, REMS emerges as a feasible, safe, and economically advantageous tool for early screening in rural contexts. Its simplicity, portability, and cost-effectiveness make it an ideal complement—not a replacement—to DXA.

The results suggest how improving OP awareness in rural areas with screening campaigns, as well as being helpful for the people to assess their bone status, can improve access to preventive care in low-income areas. Even if further longitudinal and comparative studies are needed to confirm its prognostic value and optimize its integration into national screening programs aimed at reducing inequities in bone health, REMS could help overcome diagnostic disparities in the perspective of a community-based OP care model.

## Ethical statement

This research presents an accurate account of the work performed, all data presented are accurate and methodologies detailed enough to permit others to replicate the work.

This manuscript represents entirely original works and or if work and/or words of others have been used, that this has been appropriately cited or quoted and permission has been obtained where necessary.

This material has not been published in whole or in part elsewhere.

The manuscript is not currently being considered for publication in another journal.

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All authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

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All authors have read and agreed to the published version of the manuscript."

## Human and animal right

All patients gave informed consent prior to being included in the study. All procedures involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments. This paper did not need the ethical committee's approval.

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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.

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