Non-linear mixed effect models for mechanical trials of biomaterials

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Abstract

Pelvic organ prolapse (POP) is a disorder that afflicts women of all ages, severely affecting their life quality. This condition can be characterized by the drop of intrapelvic organs. Following the growing interest of scientific community in this issue, this study intends to describe the mechanical behavior of vaginal tissue, which is one of the organs most affected. For this purpose, we implemented a nonlinear mixed effect (NLME) model to study the dynamics present in the uniaxial tensile tests performed on vaginal tissues. Where the prediction of the stress as a dependency of stretch is an innovative proposal. We also explore the possibility of implementing the model using attributes that are easily obtained. This thesis resorted to a database of the results of several experiments applied to tissue samples and patients co-variables. Here we show that a correlation exists between the patients co-variables and the mechanical properties of their tissues. According to results obtained, on average, the age and the number of children are associated with an increment of the maximum stress registered in the tests. In this thesis, we also proposed an iterative process to identify and remove outliers. When applied to our dataset, this process reduced the error and variance of the random effects by 11% and 45%, respectively with an exclusion of 17% of the samples. This innovative method has further relevance since it can be easily applied to all areas where the NLME models are proved to be useful. The application of the suggested approaches to other databases could help the development of new means of diagnosis and treatment.

Keywords: NLME, POP, Random effects, Vaginal tissue, Outliers

1. Introduction

Pelvic organ prolapse (POP) is a common, non-life-threatening condition-affecting woman of all ages [1]. Although the mortality associated with these disorders is low, the quality of life and perception of the patients own body are severely affected [2].

The exact percentage of the population that suffer of such disease is unknown, but recent studies estimated that 25% of adult women in the United States have more than one pelvic floor disorder, and 1 in 4 women will undergo surgery for stress urinary incontinence or pelvic organ prolapse during their lifetime. Routine gynecological examinations reveal evidence of pelvic organ prolapse in up to 50% of adult women [3]. In the United States alone, the costs for POP and incontinence surgeries are more than $1 billion per year. Unfortunately, this number is expected to double in the next 30 years, as a result of the increasing age and the changes into the lifestyle that will be reflected in the number of women that resorts to the healthcare system [4].

Despite the number of reports devoted to investigating these diseases being steadily increasing, there is a paucity of epidemiological studies of the natural history of pelvic disorders, making it difficult to understand their pathophysiology deeply [2]. Reliable models can be useful tools to perform objective and specific evaluation of pelvis [5]. Hence, identifying the impact of different surgical treatments and determining the most precise ways of preventing the disease is the fundamental motivation of the present work.

This study introduces a new approach, where the purpose is to predict the stress in a uniaxial tensile test as a dependency of the stretch.Furthermore, it is possible to include covariates and evaluate the association between them and the resulting biomechanical properties. In this work, we explore the possibility of implementing a NLME model using attributes that are intuitive and easy to obtain.

2. Medical and mechanical context of prolapsed tissue

The present section addresses the fundamental biomechanics background that supports this work.

Several reports characterize this POP as the drop of intrapelvic organs such as the uterus, bladder, urethra and rectum. Deficiencies in the pelvic support system are the cause of this disorder [6]. Which, most of the times, is characterized by a sensation of a bulge or protrusion, seeing or feeling a bulge or protrusion, pressure and heaviness [7].

In the literature, several studies indicate that POP is a multi-factorial problem. Vaginal birth is presumed to be one of the most relevant risk factors especially if forceps were used [8]. The risk of having prolapse surgery was more than doubled after forceps delivery compared with both vacuum and non-instrumental delivery, and more than 20 times that of cesarean [9]. Age is considered to be the only non-modifiable risk factor that is inherently and directly related to the incidence of POP. According to published reports, POP occurs mostly in older women, where the average age of patients with prolapse is 62 years old. Furthermore, the associated risk of developing POP becomes higher every year [2]. In another context, some evidences show that menopausal women who took standard doses of hormone or estrogen replacement for 5 or more years were less likely to develop pelvic floor disorders [10].

The definition of the biomechanical properties of vaginal tissue is an essential step in the modeling of the pelvic cavity, which is required to understand the physiopathology of prolapse [5]. Therefore uniaxial tension test becomes a very valuable tool to describe the disease and to understand what variables influence the degradation of tissue.

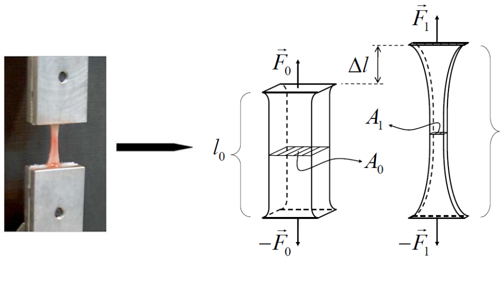

Figure 1: Uniaxial tension test for assessment of biomechanical properties of vaginal tissue. The reference configuration (F_1, L_0,A_0) is changed during the mechanical test (F_2, L_0,A_1 and $\triangle$L). (Adapted from [11][12])

In a uniaxial tension test, the quantities measured are the force applied, F, the elongation of the gauge, $\triangle$L and the cross-sectional area,$\triangle$A.

Generally in the literature, due to simplification purposes, these type of materials are considered incompressible. Therefore we are able to apply the equation of volume preservation during the test, which can be described as:

$$V_0 = V_1 \Leftrightarrow A_0 \cdot l_0 = A_1 \cdot l_1 \quad (1)$$

Data is then manipulated in order to take into account the sample geometry. The elongation measurement is used to calculate the engineering stretch λ:

$$\lambda = \frac{l_0 + \triangle l}{L_0} \quad (2)$$

To achieve the engineering stress,σ, we employ the force measurement, resorting to the following equation:

$$\sigma = \frac{F}{A} \quad (3)$$

Constitutive modeling of soft tissues has been an area of extensive research in the last few years([13, 14]). In a recent paper, Martins et al [15] modeled vaginal tissue as a hyperelastic material and led to the proposal of a strain energy function (SEF) adapted to the tissue structure. This work was followed by an investigation concerning the damage mechanisms in the tissue due to the application of stresses outside the physiological range analyzed in some mechanical test [16].

3. Nonlinear mixed-effects models

Nonlinear mixed-effect modeling is mainly applied to longitudinal data. For repeated measures it can be thought of as a hierarchical model involving both fixed-effects associated with the population parameters and random-effects accounting for unexplained inter- and intra-individual variability [17].

Possible applications for this models are vast. In many time-series investigations, including those on human immunodeficiency virus (HIV) viral dynamics, pharmacokinetic reports, and studies of growth and decline analysis, it is becoming one of the most popular type of models [18]. Nowadays, they are a routine tool for the analysis of pharmacokinetic and pharmacodynamic data obtained in clinical trials during drug development [19].

As described, the literature on NLME models shows a wide variety of applications, but to our knowledge, all the models have been developed as a function of time. This study introduces a new approach, where the purpose is to predict the stress in a uniaxial tensile test as a dependency of the stretch. Stretch, our "time", is also continuous, monotone increasing function, and always positive. Therefore, all the key conditions demonstrate the feasibility of this new proposal. Furthermore, it is possible to include covariates and evaluate the association between them and the resulting biomechanical properties.

In this work, we explore the possibility of implementing a NLME model using attributes that are intuitive and easy to obtain. Patient features used were only the ones described in the literature as being potential risk factors of pelvic organ prolapse.

The nonlinear mixed effects model is a natural generalization of linear mixed effects model. Let y_{ij} denote the jth measurement of the response. In this case, it represents the stress resultant for a certain value of stretch, under the condition $t_{ij}, j = 1, ..., n_i$. Each individual has a vector of their characteristics defined by a_i, which do not change over the measurements, for example, age, parity and hormonal treatments. Letting $y_i = (y_i, u_i, a_i)$ be independent across i, reflecting the belief that individuals are unrelated. In this work perspective, the NLME model can be written into a two-stage hierarchical form as follows:

Stage 1: Intra-subject variation

$$\sigma_{ij} = f(\lambda_{ij}, \beta_i) + e_{ij}, \quad j = 1, ..., n_i. \tag{4}$$

In equation 4, $f(.)$ represents a function governing within-individual behavior and it depends on a $(p \times 1)$ vector of parameters, β_i, specific to the individual i. The function $f(x_{ij}, \beta_i)$ represents what happens on average for all possible realizations of actual response trajectory and measurement error that could arise if subject i is observed. Generally, the function f is incapable of include all the process dynamics, meaning that the real response, followed by the dotted line, is always different than the predicted output. Parameter e_{ij} represents the absolute error between the actual and the predicted response. It is commonly assumed that intra-individual deviations $e_{ij} = y_{ij} - f(x_{ij}, \beta_i)$ have mean equal to zero, $E(e_{ij}|, \beta_i) = 0$.

Stage 2: Inter-subject variation

$$\beta_i = d(a_i, \beta, b_i), \quad i = 1, ..., m, \tag{5}$$

Where d is a p-dimensional function depending on an $(r \times 1)$ vector of fixed effects,β, and a $(k \times 1)$ vector of random effects b_i associated with individual i. Equation 5 describes how β_i varies among individuals, due to explained systematics correlation with individual characteristics in a_i and to "unexplained" variation in the population of individuals, represented by b_i. Usually it is assumed that the distribution of b_i is independent of a_i, with $E(b_i|a_i) = E(b_i) = 0$ and $\text{var}(b_i|a_i) = \text{var}(b_i) = D$. Parameter D is an unstructured covariance matrix that is the same for all i. D represents the magnitude of "unexplained" variation in the elements β_i and associations among them. A standard such assumption is $b_i \sim \mathcal{N}(0, D)$, where D is the magnitude of the random effect.

As related before, the choice of the structure of $f(\lambda_{ij}, \beta_i)$ is one of the most important factors of the model's success. To reproduce the behavior of $\sigma(\lambda)$ accurately, we tested several equations in the fittings. It is worth mentioning that the physical meaning of the individual parameters, $\beta_i = (\beta_{1i}, \beta_{2i}, ..., \beta_{pi}) = (c_{1i}, c_{2i}, c_{pi})$, depends on the equation structure, where the vector c_{ji} contains the constants to be fitted for each individual. For this concrete case of study, the proposed equations is as follows:

$$f(.) = c_{1i} \cdot e^{-(\frac{\lambda_i - c_{2i}}{c_{3i}})^2} \tag{6}$$

Non-linear mixed effects models rely on the level of correlation between the individual features and the response that is supposed to predict.

In this problem context, theoretically inferring the vector β_i is hard to achieve, hence the process of defining β_i vector is fundamentally empirical. After the definition of $f(.)$ function, its parameters should be related to the co-variables of each patient. As assumed before, $\beta_i = (c_{1i}, c_{2i}, c_{3i}) = d(a_i, \beta, b_i)$, where d, in this case, is a 3-dimensional function since $f(.)$ has three parameters. We should be aware that the definition of $d(.)$ directly depends on the $f(.)$ function chosen before. The proposed structures for the $d(.)$ are the following: Polynomial combination of co-variables with a defined maximum degree; Assuming that the co-variables do not have any influence.

The vector of fixed parameters, β, is given by the concatenation of all vectors of constants involved in the p-dimensional vector d.

As demonstrated in this section, the success of model predictions depends on a large variety of factors. In this type of models, the choices of f and d are the most critical decisions. Due to the lack of knowledge about which variables have influence and the impossibility of theoretical deducing the equations that govern the mechanical behavior of human tissues, we are only able to calculate local solutions inside the spectrum of structure types tested. Therefore, the process of defining $f(.)$ and $d(.)$ functions can be considered a heuristic algorithm.

4. Outliers detection in time series

Having in mind that this study is working with real data, it is essential to introduce the outliers subject. Hence, this section will explain the relevance of this topic on the overall study, beginning with the presentation of the outliers definition. An outlier is defined as an observation that discrepant with the remaining data. In other words, is an element that does not fit the general pattern of the data [20].

The subject addressed is a particular case of outliers universe because it is only related to time series. They can be expressed as a set of data points indexed in time order, where the observations can be or not equally spaced in time. As explained in the previous sections, the graphs of the uniaxial tensile tests are not in order of time, but of stretch. So it is necessary to interpret the stretch as time.

As described before, in experimental studies there is always the possibility of having data subject to errors. From the previously enumerated sources, the failures originated in the tests measurements are worth to being mention again.

The processes of identifying outliers are a topic of growing interest for several reasons. Being uncorrelated with the remaining observations, they can have a significant influence on the overall results, leading the analysis to incorrect conclusions. Therefore it is of the utmost importance to previously identify and remove them to develop better analyses, models and achieve useful conclusions.

In this thesis, a new approach to identify and remove outliers from a dataset is also proposed, in order to improve the parameter estimation results. In order to understand the introduction of this method, we have to realize that a NLME model includes the random effect component, b_i and that can be interpreted as the unexplained error that a sample has compared to the estimated general pattern. Assuming that, after the model is developed, the outliers have more probability of having greater error than the rest of data, we can deduce that the samples with a higher b have the highest chances of being outliers. The innovative part of this approach consists in relating random effects with outliers. Hence, this process is fundamentally empirical because we need to create the model to analyze the random effects of each data point. Therefore, this approach does not guarantee that the solution obtained corresponds to the optimal solution.

5. Implementation

The process of achieving the optimal model implies experimenting several solutions until the best results are obtained. Hence, the development of a program, fully automatic, that allows the creation of models with different characteristics is proposed. Such as $f(.)$ and $d(.)$ structures, individual co-variables.

The program is implemented in the MATLAB platform. We chose this platform because there are available several commands that facilitate the process of developing the models. MATLAB is also one of the most powerful computational tools to solve engineering problems.

The program implemented is described in Figure 2. First, we proceed to the data processing which is a fundamental phase in the model development. This block transforms and structures the data so the model can easily interpret them.

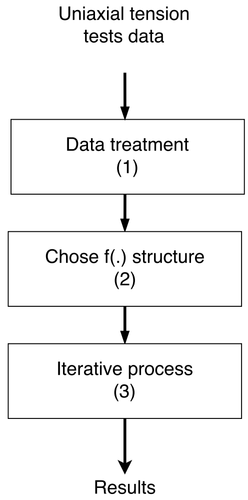

Figure 2: Iterative process flow chart

Finished the previously step, next block's main goal is to choose the more suitable $f(.)$ structure. Having the stress/stretch data points, it is found the equation which better fits the data. The fittings are performed resorting to the MATLAB command *fit*.

For the following phase, an innovative algorithm was implemented in order to identify and remove outliers from a dataset. This algorithm is essentially a set of iterative cycles that determine the points that are repeatedly poorly explained by the models, as outliers. These discrepant observations are chosen based on the magnitude of their random effects. It should be noted that inside of the current block the fittings of the patient co-variables with the $f(.)$ are performed. As explained previously, we experimented several structures for the $d(.)$ equations. For the polynomial form, to calculate the parameters of the equations, we use the MATLAB command *fitlm*. The developed method's main goal is to improve the model accuracy.

6. Technical procedure to evaluate tissues

To fulfill this thesis's objectives, we require a dataset that provides the information relevant for the model implementation. Knowing that our focus is on the system's mathematical description, we were not responsible for collecting the dataset. In the scope of a collaboration between the Faculties of Engineering and Medicine of the University of Porto and *Hospital de S. Joao do Porto*, patient's vaginal tissues were collected and submitted to mechanical tests.In the present work, we will use the database already created with the biomechanical tests.

Experimental vaginal tissues were gathered from women submitted to surgery for POP. The study was conducted following the Ethical Research Ethics Committee guidelines of *Hospital de S. Joao do Porto*. Patients give their consent for the following studies. All women were previously evaluated by several factors, such as history, physical examination and some by urodynamic study. The severity of POP symptoms was previously evaluated as well. To be admitted in the present study, women must had vaginal prolapse at least stage 2 of POP-Q [11].

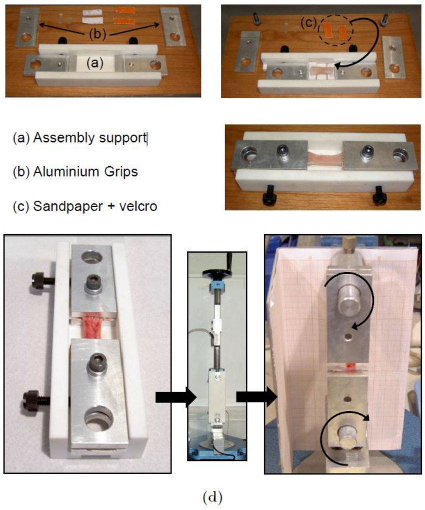

Figure 3: Sample preparation. (d) Mounting the sample in the test rig. (Adapted from [12])

Once removed the tissues, the mechanical tests were performed on each valid sample. Accordingly with Martins et al. [21], the sample tissues were fixed on the testing machine utilizing an assembly support. It should be noted that the device was developed by Martins and colleagues to perform uniaxial tensile tests on soft tissues. The figure 3 shows the system where the sample mechanical properties are evaluated. Geometric properties were measured using a digital image analysis.

7. Data analysis

This section is dedicated to describing the real data gathered. We will present some mean full statistics that help the understanding of the influence of the data in the results obtained. The information required to implement a NLME model includes the observations of the uniaxial tensile tests and the co-variables of every patient.

The co-variables gathered and the transformation made to improve the models accuracy is summarized in Figure 4. At this point, it is important to understand the several reasons why the transformations were performed. The number of variables is reduced allowing a faster convergence and decreases the possibility of over-fitting. Over-fitting is a relevant problem because, as will be explained later, the dataset is not very large. Finally, we also assumed that the birth by cesarean does not influence the mechanical properties of vaginal tissue.

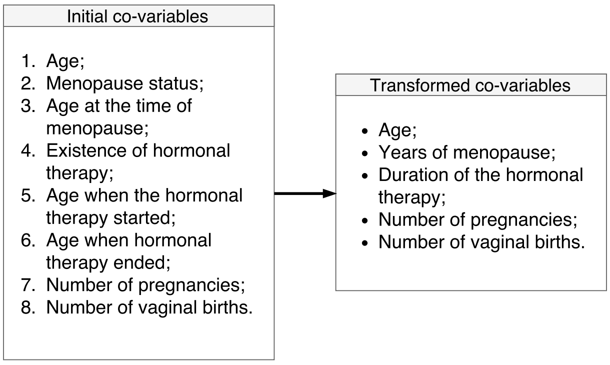

Figure 4: Variables transformation

Table 1: Statistical description of the patient co-variables.

	Age [Year]	YM [Year]	DHT [Year]	NP [-]	NVB [-]
Mean	63,6	15,5	0,9	3,4	3,0
SD	11,7	12,1	1,9	2,0	1,8
Min	38,0	0,0	0,0	0,0	0,0
Max	84,0	43,0	6,0	10,0	9,0

Table 1 summarizes the statistics regarding the co-variables. For each one, the parameters arithmetic mean, standard deviation, minimum and maximum are presented. The dataset is composed by 27 patients with complete information. Every patient has at least one valid representation. In total, this study resorts to 89 valid samples.In average the sample's maximum stress is 545.9 KPa, the minimum is 139.4 KPa and the maximum is 1565.9 KPa.

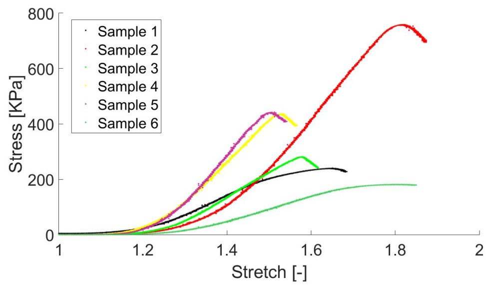

Figure 5: Tests on samples from the same patient

Finally, an example of the uniaxial tensile test applied to several samples of the same patient is presented. Figure 5 shows the results of uniaxial tensile tests applied on six samples from the Patient 17.

8. Results

This section is dedicated to presentation of the results obtained by applying NLME models on uniaxial tensile tests data of vaginal tissue. In the first chapters, it was explained the reasons behind the application of this type of models, in patients' tissues. The primary objective is to predict mechanical properties of tissues based on patients individual characteristics. The indicators obtained during the modeling process shall quantify the degree of certainty of predictions given by the developed models. The choice of the MATLAB command, used to perform the fittings, was decided based on several factors, such as convergence speed, reliability, and accuracy.

As explained in the previous sections, the equation used to describe the uniaxial tensile test observations is the Gauss equation.

Firstly, the algorithm assumes that each sample is independent. Only in the patient model estimation is assumed that samples from the same individual are correlated. So we executed fittings for both situations. The fittings were performed resorting to the MATLAB command *fit*.

Table 2 summarizes the fittings results regarding the constants of the $f(.)$ equation. The statistics presented are very relevant to evaluate the model accuracy. Two relevant indicators, the RMSE and the R-square are presented. While the first measures the effective error between observations and the predicted value, the second quantifies the proportion of the variance in the output that is anticipated by the input. Regarding the fittings on individual samples, the average error is minimal, and the R-square is one in almost every sample. In the worst case, RMSE is equal to 21.83, and the coefficient of determination is equal to 0.88. Even in this situation, the fitting has a satisfactory quality. From the results, we can conclude that the Gauss equation makes an excellent approximation of the observations. It should be noted that we conclude the significance of the error by comparing it with values of the constants c_i.

In the same Table, the information concerning the patients fittings is also described. In other words, the samples are grouped by patients before we execute the estimations. The results presented show that the approximations are worst than in the previous situation. In average, the RMSE is nearly eleven times higher, and the R-square is about 27% smaller. The fittings are suitable when applied to a single test, but when we apply to several samples from the same patient the results get worst. Due to the high variability of the tests intra-individual, the patients with a greater number of samples have poorest fittings quality.

Previously, it was explained that the MATLAB command *fitlm* is the one that better satisfies this study's requirements. Since we were not able to find an explicit dependency of the function, this study decided to assume a polynomial structure for the $d(.)$ vector equation.

After several experiments, the co-variables that will be used are Age, YM and NVB. This is the combination that guarantees the best results. We also decided that the algorithm must leave at least one test per patient, since we have a small dataset, we want to avoid removing individuals.

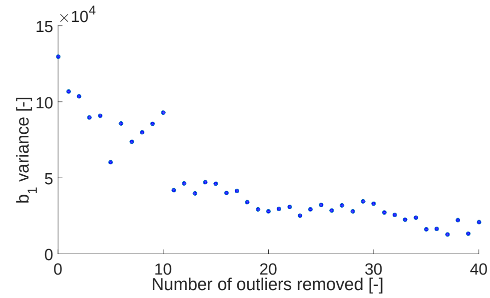

Figure 6: Variation of the random effect, b_1, variance with the number of outliers removed.

When the iterative process finishes, we have to decide how many outliers are to be removed. This decision is taken based on the variance of random effects. Figure 6 only illustrates how the parameter c_1 varies with the number of outliers removed since the other parameters follow the same behavior. The samples to be removed are ordered by the number of times that were considered outliers by the algorithm. The variance of all random effects diminishes steadily as the algorithm is removing more samples. Analyzing the graph, we can see that the removal of the firsts 15 outliers had a significant impact on the variance but after that point, the variance variation reaches a horizontal asymptote. In the three cases, the variation drops around 80% which means that removing erroneous tests allows the model to explain the remaining data better.

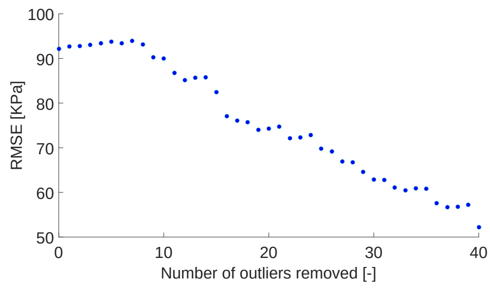

Figure 7: Variation of the model RMSE with the number of outliers removed.

The figure 7 describes the RMSE variation when the algorithm removes the outliers. The error follows the predictable pattern: it starts equal to 91 and stays constant until ten samples are eliminated. After that point it diminishes gradually since the

Table 2: Statistical description of samples and patients fittings.

	Patient fittings					Sample fittings				
	c_1 [-]	c_2 [-]	c_3 [-]	RMSE [KPa]	R-square [-]	c_1 [-]	c_2 [-]	c_3 [-]	RMSE [KPa]	R-square [-]
Mean	671,1	2,1	0,5	78,0	0,8	544,9	1,9	0,4	7,2	1,0
SD	387,0	0,3	0,2	41,4	0,1	311,0	0,3	0,1	4,4	0,0
Min	153,2	1,6	0,3	4,6	0,5	126,0	1,5	0,2	1,6	0,9
Max	1537,9	3,0	1,0	157,8	1,0	1615,4	2,9	0,7	21,8	1,0

model has fewer samples to fit, thus it can adjust more easily. When the iterative process finishes, we have to decide how many outliers are to be removed. This decision was also taken based on the variance of random effects, so it was decided to remove 15 outliers. This decision took into account the balance between the lost of information and the model accuracy. If we proceeded to exclude more samples, the results would be better, but we were adjusting the dataset to the pretended results excessively.

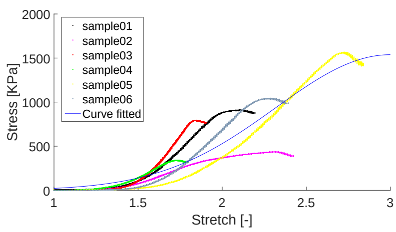

Figure 8: Samples of the Patient 31.

Patient 31 is the one that had more tests considered invalid. The tests applied to the samples from the referred patient are presented in Figure 8. The results show a substantial discrepancy of their mechanical behavior so it is reasonable that the algorithm considers several samples as outliers.

After analyzing the model results, we will describe the model obtained. The estimated covariance matrix is expressed by:

$$PSI = \begin{bmatrix} 4.4 \times 10^3 & 0 & 0 \\ 0 & 2.6 \times 10^{-2} & 0 \\ 0 & 0 & 5.2 \times 10^{-3} \end{bmatrix}$$

The variance of each random effect is very discrepant because of the scale of the parameter with each random effect is associated. The matrix agrees with the values expressed in Figure 6.

In order to quantify the influence of the outliers removal, we compare the results of the first model containing all the samples and the final model without 15 outliers. The influence of the excluding process is expressed in Table 3. It is shown the error and the variance of the three random effects. All the indicators show that the final is better than the

Table 3: Statistical analysis of the initial model and the final model

	Final model	First model	Variation [%]
RMSE [KPa]	82,20	91,90	-10,55
b_1 SD [-]	212,60	360,20	-40,98
b_2 SD [-]	0,16	0,28	-42,09
b_3 SD [-]	0,07	0,15	-52,63

Table 4: Statistical analysis of the simple model and the final model

	Final model	Simple model	Variation [%]
RMSE [KPa]	82,20	97,52	-15,71
b_1 SD [-]	212,60	395,50	-46,25
b_2 SD [-]	0,16	0,32	-49,69
b_3 SD [-]	0,07	0,18	-60,00

first one. The RMSE is reduced by 11%, which represents a slight improvement. The variance of b_1, b_2 and b_3 is reduced by 41%, 42% and 53% respectively. The variation in percentage illustrates that the iterative process is a suitable tool to improve the NLME models. Excluding few samples, the model performance is increased. If we move the analysis to the absolutes values of the final model, we conclude that the error is still large. The model was improved, but it cannot yet describe the system by the fixed effect part. Therefore the variance of the random effects is very high.

To evaluate the importance of the co-variables in the model implemented, we created a model that does not include them. When we compare the performance of both models, it is possible to infer if the individual's characteristics add relevant information to the model.

Table 4 summarizes the error and variance of each model. As can be seen, all indicators are better the final model. The RMSE is reduced by 16%, which represents an improvement. The variance of b_1, b_2 and b_3 is reduced by 46%, 50% and 60% respectively. Which means that the co-variables have a beneficial influence on the results. We should have in mind that the simple model utilize fewer parameters to describe the dataset and this results in less over-fitting problems. When we work with a small dataset, this issue is substantially increased. In this perspective, it has a slight advantage compared to the final that resorts to much more complicated equations and try to fit several variables recurring to just 72 samples. Therefore, with a more significant dataset, probably the difference between the two models would be substantial.

After an analysis of the $d(.)$ equations which describe the association between the patient co-variables and parameters of the $f(.)$ function, we note that the parameter c_2 is the simplest one. It only requires the age to be described. The other parameters need more terms and require more co-variables to be explained by an equation. From the equations obtained, the age effects all parameters. Menopause and number of vaginal births influence c_1 and c_2. It should be noted that we limited the size of the polynomial functions. Without counting with the random effects, their maximum size is four terms.

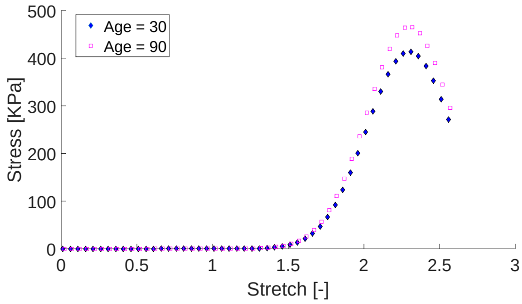

Figure 9: Influence of the Age on the predicted uniaxial tensile tests. Both samples have NVB = 3 and YM = 15.5.

After estimating the equations that relate the co-variables with the uniaxial tensile tests, it is essential to represent their behavior using example cases. From Figure 9, we can see the influence of age in the equation obtained. There are expressed two predictions in the graph. The blue dotted line corresponds to a 30 years old and the other to a woman 90 years old. The oldest example has the highest maximum stress. This parameter is directly related with the c_1 that goes from 414 to 466, meaning a variation around 12%. The remaining parameters almost do not change.

Figure 10 illustrates how the curves vary with menopause. The difference in the curves constants does not even reach the 2%. Hence this co-variable show a minimal influence on the results. A possible cause for this outcome is the correlation that exists between the menopause and age. It is possible that the model did not give relevance to menopause

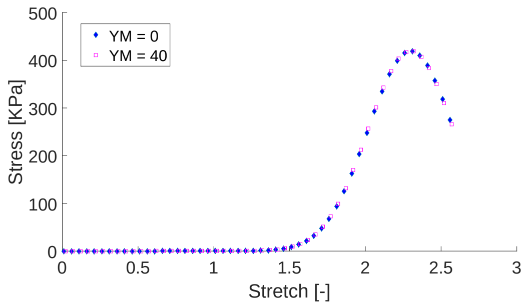

Figure 10: Influence of the YM on the predicted uniaxial tensile tests. Both samples have NVB = 3 and Age = 63.6.

because the age already adds a similar information. However, in average using Menopause produced better results than without it.

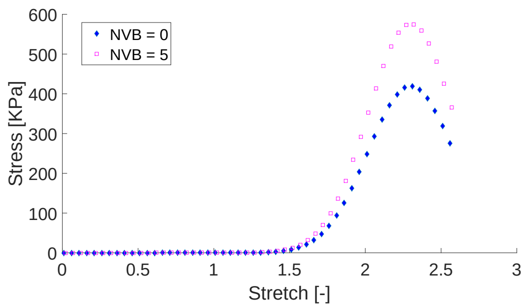

Figure 11: Influence of the NVB on the predicted uniaxial tensile tests. Both samples have YM = 15.5 and Age = 63.6.

Figure 11 presents the relation between NVB and the estimated Gauss equation. It is clear that this co-variable has a relevant influence on the curve. There are expressed two examples with different values of NVB. On one its value is 0 and on the other it is 5. The parameter C1 value goes from 419 to 456, meaning a variation around 8%. So we can infer that the number of vaginal births increases the maximum stress of the uniaxial tensile test. The others parameters do not have significant changes in their values.

It is very relevant to discuss the significance of the co-variables expressed in the previous figures. The model equations provide an excellent base to conclude which ones affect the tests made. Age and NVB are the individual characteristics that have the most prominent relevance. Mainly, they influence the curves maximum stress. In average, older women and with more children by natural childbirth have higher maximum stress. The other parameters of the Gauss equation appear to do not be affected by them.

9. Conclusions

Following an increasing interest in the POP disorder, this thesis investigated the connection between patients characteristics and their vaginal tissues' mechanical properties. The possibility of describing the uniaxial tensile tests applying NLME models was proposed and tested. In this scope, we developed a new approach to describe the dataset using the referred model and to exclude samples considered as being outliers.

We worked on a dataset composed of numerous patients, each one containing several test results. In a first approach, this study tried to approximate the tests observations to a Gauss equation. The fittings results of each sample individually were very accurate, since their RMSE was low. When the process was performed to the data grouped per patient, the errors substantially increased due to the high intra-individual variability. Across this thesis, several patients were presented as examples of the inconstancy of samples that were supposed to be correlated.

A NLME model assumes that there is an association between the individual co-variables and the parameters of the $f(.)$ function. After we performed the fittings, we analyzed the results and concluded that not all co-variables have the same influence on the parameters. The most relevant characteristics are the age and the number of natural childbirths. The existence of hormonal therapy, menopause and the number of pregnancies did not show signs of being significant in the tests results. Several experiences recurring to different functions were performed until we concluded that, in a mathematical form, this correlation is better described with a polynomial equation.

The model success is mainly controlled by the information that we use as an input. When the model was implemented, it was clear that some samples diverged from the patient's general pattern. To surpass this difficulties we developed an iterative process to identify and exclude samples supposed to be outliers. In the dataset, the removal of 15 samples reduced the variance of the random effects more than 40%. The error also was improved, dropping around 10%. We should emphasize that we were conservative in deciding the number of samples to be removed. If more tests were excluded, the results improvement would be more significant.

Concerning the primary purpose of this thesis, we concluded that there is some evidence that suggests that the patient is associated with the mechanical properties of vaginal tissues. The final model's equations support the theory that age and number of natural childbirths actively affect the maximum stress observed in the tests. Following the results obtained, we can speculate that when the women get older, their vaginal tissues reach higher values of stress during the tests, although we cannot infer that from this type of cross-sectional study. The same applies to the number of normal childbirths.

Although the trends observed, the model did not describe the data with the precision expected due to various constraints. The number of samples was proved to be insufficient to achieve better results. Aware of the need to estimate several parameters, this factor was the main limitation of this thesis, since the require estimations, we need a statistically significant number of samples to avoid overfitting problems. Another restriction factor was the high intra-individual variability that distorts the patients' fittings.

Reviewing the work accomplished, we conclude that this thesis made a few steps to improve the knowledge about the POP disorder. We concluded that some individual characteristics influence the tissues mechanical properties, which can promote the development of diagnosis means and treatments methods.

10. Future Work

In the future, the NLME models should be applied to different datasets of mechanical tests on tissue to perceive if the results were affected by the quality of our data. Finally, we consider that applying the iterative process in other application can be proved to be very useful. This method was developed to not be restricted to our dataset. Therefore, applying it to another context is relatively easy to perform and has the potential to improve the results considerably. This approach is entirely innovative, so there are many areas of interest to apply the algorithm.

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